Amelioration of acidity in fresh waters: Individual to ecosystem level responses

Gareth Brooks Jenkins

School of Biological and Chemical Sciences

Queen Mary University of London

A thesis submitted to the University of London for the degree of Doctor of Philosophy

February 2015

Statement of originality

I, Gareth Brooks Jenkins, confirm that the research included within this thesis is my own work or that where it has been carried out in collaboration with, or supported by others, that this is duly acknowledged below and my contribution indicated. Previously published material is also acknowledged below.

I attest that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge break any UK law, infringe any third party's copyright or other Intellectual Property Right, or contain any confidential material.

I accept that the College has the right to use plagiarism detection software to check the electronic version of the thesis.

I confirm that this thesis has not been previously submitted for the award of a degree by this or any other university.

The copyright of this thesis rests with the author and no quotation from it or information derived from it may be published without the prior written consent of the author.

Signature:

Date:

Collaborations

Chapter Two: Dr Björn Rall provided advice on functional response analysis. Pablo Rodriguez Lozano and Thomas Bruce completed part of these experiments under my supervision, on a PhD placement from the University of Barcelona and a QMUL undergraduate project respectively. I completed the majority of the experiments myself, as well as the entire analysis and writing of the chapter.

Chapter Four: Professor Alan Hildrew, Professor Colin Townsend, Dr Guy Woodward and Dr Katrin Layer provided historical data. I reformatted and reanalysed these data, combining them with novel laboratory experiments before writing the chapter.

Publications

Chapter Four: Layer K, Hildrew AG, **Jenkins GB**, Riede JO, Rossiter SJ, Townsend CR, Woodward G (2011) Long term dynamics of a well-characterised food web: Four decades of acidification and recovery in the Broadstone Stream model system, *Advances in Ecological Research*, **44**, 69-117

Chapter Five: **Jenkins GB**, Woodward G, Hildrew AG (2013) Long-term amelioration of acidity accelerates decomposition in headwater streams, *Global Change Biology*, **19**, 1100-1106.

Abstract

The extensive acidification of fresh waters across Europe and North America during the 20th century led to extensive and widespread loss of species, which fundamentally changed the structure of resident communities. While attempts to limit or halt acidifying emissions have been successful in reversing the chemical consequences of acidification, any corresponding biological recovery has been patchy at best. The main aim of this thesis was to investigate potential ecological constraints on this biological recovery, ranging from interactions between individuals to ecosystem-level processes, using a model stream system that has been extensively studied for over 40 years. I used a combination of long-term survey data and experiments, both in the field and the laboratory, to provide evidence that the lag in recovery of acidified aquatic communities is due to intrinsic resistance to re-colonisation by acid-sensitive species, as a result of both direct and indirect interactions between predators and prey. However, there is also evidence of recovery at an ecosystem level, with rates of microbial decomposition increasing – a key link in the transfer of energy to higher trophic levels, which could be facilitating population increases in large-bodied predators. These findings increase our understanding of the drivers which govern the structure and function of ecological networks in response to an important climatic stressor. This is especially relevant given the rapid industrialisation of countries such as India and China, which could well soon experience anthropogenic acidification on a significant scale.

Acknowledgements

Firstly, I'd like to acknowledge my supervisors, Dr Guy Woodward and Professor Alan Hildrew for their support and guidance, and for never failing to provide feedback to any questions I had. I'd especially like to thank them for encouraging me to get involved in other side-projects above and beyond my thesis, which really gave me the chance to get as much out of my time at QMUL as I could. The chance to go on fieldwork to places such as Iceland, the Scottish Highlands and Snowdonia was brilliant. Of course, I also ended up in places like Welshpool and Paisley, but you can't have everything.

Dr Jonathan Grey stepped in to help when I needed a third supervisor towards the end of my PhD, which I'm grateful for. Dr Steve Rossiter acted as my panel chair throughout the PhD and always provided useful and constructive comments to all my reports.

This thesis has, at times, involved statistical programming. Thankfully, I know some very smart chaps who have been only too happy to help out. Dr Björn Rall provided invaluable assistance and advice when constructing functional response models. Likewise, both Dr Lawrence Hudson and Dr Eoin O'Gorman have always unfailingly answered questions about R on numerous occasions, which was very kind of both of them.

As any ecology PhD student will tell you, it's impossible to get anything done in the field without the generous help of others. Flick Shelley, Sean O'Brien, Sarah Tuffin, Clare Gray, Eoin O'Gorman and Bruno Gallo were some of the many people that helped me out, so if I've forgotten you, I'm sorry. I'm not that sorry actually, you've already done the work. But thanks anyway.

In the postgraduate office, there's been a rotating cast of splendid folk whose company has made it a fantastic four years. So – Michelle Jackson, Rick Hayes, Muzungu Sanders, Gregory Carey,

Joanne Littlefair, Rebecca Stewart and Dozza Eve Pichler – thanks for everything. There's always time for just the one, right?

Last but definitely not least - special thanks for all the support over the years from Mum, Dad, and Siân. I couldn't have done it without you three and I'm very grateful.

This work was generously supported by the Natural Environmental Council, via a Doctoral Training Grant (# NE/1528069/1).

Table of Contents

Statement of originality……..…..….2

Collaborations/publications…….…….....3

Abstract…….4

Acknowledgements…….…...5

Table of contents…….……...7

List of tables…….....…10

List of figures…….…….…..11

Chapter One: General Introduction…….…..….13

Anthropogenic acidification of fresh waters……..……...13

Chemical and biological recovery from acidification……...…….16

Food webs: Function, stability and structure……..…….…..20

Broadstone Stream and the Ashdown Forest……..….24

Aims and thesis structure……...……....27

Chapter Two: Simulating species interactions in recovering communities: functional responses of acid-tolerant predators to prey characteristic of acidic and circumneutral streams…….……31

Abstract…….....…..31

Introduction……..….....32

Methods…….……..38

Study Sites……...…38

Laboratory experiments……...……..…….39

Statistical analysis……..42

Results…….....44

Functional responses……..…….….44

Prey mortality……...…….....45

Temperature response……...…….46

Tables and figures……...….47

Discussion……..……..58

Chapter Three: Simulating indirect interactions between acid-tolerant and acid-sensitive species…….…62

Abstract……..…….62

Introduction…….....…..63

Methods…….…..…68

Study site……..…..…..68

Laboratory experiments…….....……..69

Statistical analyses…….....……..72

Results……...……...…74

Tables and figures…….……..……76

Discussion……82

Chapter Four: Effects of long-term amelioration of acidification and invasion by a vertebrate predator on a well-characterised food web……...……...87

Abstract……..…….…87

Introduction……..……..88

Methods……...……93

Study site……...……..93

Characterising and constructing the food webs……...94

Laboratory experiments……..……96

Statistical analyses……..……98

Results……..……...…99

Mass-abundance patterns……...……....99

Laboratory experiments……..……...…100

Tables and figures……...……...…101

Discussion…….……107

Chapter Five: Long-term amelioration of acidity accelerates decomposition in headwater streams…….….112

Abstract……..……112

Introduction……...……113

Methods…….……116

Study site…….…..116

Environmental data…….…...117

Cellulolytic decomposition……..…..118

Statistical analyses…….……120

Results……..…..121

Tables and figures…….……123

Discussion…….…131

Chapter Six: General Discussion……..…135

References……..…141

Appendices……..……...160

List of Tables

Chapter Two

Table 2.1 Linear mixed effects model (LMEM) with restricted maximum likelihood method (REML) nested output of changes in prey mortality……...…47

Table 2.2. Parameters and standard errors (SE) estimated by the temperature dependent model……...…...48

Chapter Three

Table 3.1. Average relative crowding coefficients (*k*)……...…….76

Table 3.2. Three-way analysis of variance (ANOVA) testing for effects of prey species, density and trout kairomone on survival rates of prey……...….77

Chapter Four

Table 4.1 The Broadstone Stream food web taxa (1970s – 2000s)……..……..101

Table 4.2 Chi-squared goodness of fit test for three classes of interaction strength……102

Table 4.3 Fully factorial ANOVA testing for effects of predator species identity and presence/absence of fish kairomone on prey survival rates…….…….103

Chapter Five

Table 5.1 Values of physicochemical variables in 34 stream sites…….…..123

Table 5.2 Results of ANCOVA on temperature-adjusted winter decomposition rates with year as fixed effect and pH as covariate……...124

List of Figures

Chapter One

Figure 1.1 Theoretical depiction of Type I, II and III functional responses……..29

Figure 1.2. Schematic connectance food web of Broadstone Stream, with links coded to specific chapters of this thesis……..…….30

Chapter Two

Figure 2.1. Summary binary food web for Broadstone Stream with experimental species highlighted on conceptual food web subset…….49

Figure 2.2. Individual networks of some interconnected stream food webs from the Ashdown Forest catchment……..50

Figure 2.3. Experimental design for predator-prey interaction experiment……...….51

Figure 2.4. Functional response curves for *C.boltonii* for individual prey eaten

per predator……..52

Figure 2.5. Functional response curves for *C.boltonii* for prey biomass in milligrams eaten per predator……..…..53

Figure 2.6 Functional response curves for *S.fuliginosa* for individual prey eaten

per predator……..54

Figure 2.7 Functional response curves for *S.fuliginosa* for prey biomass in milligrams eaten per predator……..…..55

Figure 2.8 Functional response curves for *P.conspersa* for individual prey eaten

per predator……..56

Figure 2.9 Functional response curves for *P.conspersa* for prey biomass in milligrams eaten per predator……..…..57

Chapter Three

Figure 3.1 Functional response curve of *Cordulegaster boltonii–Baetis rhodani* interaction at 10°C……...…..78

Figure 3.2 Experimental design for indirect interaction study……...….79

Figure 3.3 De Wit replacement diagrams……...….80
Figure 3.4 Ratio diagrams showing relationship between log input and output ratios of the two prey species…….....81

Chapter Four

Figure 4.1 The pre- and post-invasion Broadstone Stream trivariate food webs……..…104

Figure 4.2 Individual Size Distribution (ISD) mass-abundance plots for the Broadstone Stream assemblage…….....105

Figure 4.3 Mean survival rates of two common prey species in the presence/absence of trout kairomones……..….....106

Chapter Five

Figure 5.1 Calibration between mean air and stream temperatures…….127

Figure 5.2 Average annual pH of Ashdown Forest survey stream sites for 1978/9 and 2011/12……...……..……128

Figure 5.3 Rate of decline in tensile strength for Shirley cellulose test cloth……..129

Figure 5.4 Temperature-adjusted log-transformed tensile strength loss of Shirley cellulose test cloth……..130

Figure 5.5. Comparison between Shirley and calico test cloth tensile strength loss……131

Chapter One: General Introduction

1.1 – Anthropogenic stressors in fresh waters.

Freshwater systems are of fundamental importance in providing us with resources (Ormerod *et al.* 2010), but are increasingly threatened by a suite of environmental stressors (e.g. drought, land use change, nitrification, warming). Fresh waters are particularly vulnerable due to their fragmented nature and dependence on climatic conditions for temperature control (Woodward *et al.* 2010). The rapidly increasing influence that anthropogenically-generated stress is having on natural ecosystems is an area of concern in freshwater ecology; especially given that fresh waters and their catchments can be subject to multiple stressors. These stressors affect fresh waters at all levels of organisation; from individuals up to entire catchments. In this thesis, I aim in investigate the impact of one such stressor – anthropogenic acidification – at varying levels of organisation, in order to understand how the impact of environmental stress manifests across entire ecosystems.

In the 1970 and 1980s, significant scientific attention was paid to the possible causes of surface freshwater acidification (e.g. Almer *et al.* 1974; Harvey 1980; Wright *et al.* 1980; Flower & Battarbee 1983; Ormerod *et al.* 1988), which was affecting lakes and rivers across large parts of Europe and North America (Mason 1991; Hildrew & Ormerod 1995; Driscoll *et al.* 2001). It first came to attention as a result of episodic fish deaths and declines in fish stocks, and especially those of economically important salmonids, in particular areas (Rosemund *et al.* 1992). The causes of pH decline were not initially understood, and there was a lack of any comprehensive historical data (Monteith *et al.* 2005). An early suggestion was that the land-use change was responsible. Arable farming was in decline in nations experiencing acidification and it was hypothesised (Rosenqvist 1978) that the most common alternative land use, afforestation with

conifers, was driving an accumulation of acidic humus in riparian soils, which eventually leach H+ ions into the water. However, acidification was also occurring in areas of little to no land use change (Battarbee *et al.* 1990). A separate hypothesis was that the deposition of strong acids was responsible for freshwater acidification, driven by anthropogenic industrial emissions (Flower & Battarbee 1983; Battarbee *et al.* 1988). Palaeolimnological studies of sediment cores extracted from lakes confirmed this theory, and industrial pollution of the atmosphere is now widely accepted as the primary cause of freshwater acidification in lakes (e.g. Battarbee *et al.* 1990; Birks *et al.* 1990; Hildrew 2009). Such palaeo-analyses are rarely possible in streams, due to water flow disrupting any historical sediment deposition, but emissions undoubtedly had a similar effect on streams and their biological communities (Ormerod *et al.* 1988).

Acidifying emissions, primarily sulphur and nitrogen oxides (Monteith & Evans 2001; Monteith *et al.* 2013), are produced via industrial combustion of fossil fuels. These emissions consequently release strongly acid anions into water through deposition on freshwater catchments. In well-buffered catchments, such as those underlain by chalk and other base-rich geologies, high concentrations of neutralising compounds (e.g. carbonates and bicarbonates) act to reduce the acidity in runoff (Fromm 1980). However, in poorly-buffered catchments, the acid neutralising capacity (ANC) of the soil is soon exhausted and toxic cations, in particular hydrogen ions (H^+) and labile aluminium (Al^{3+}), are released into waters. It is the toxic effects of such compounds that lead to direct physiological stress and/or death of a wide range of aquatic organisms (Baker & Schofield 1982; Schlinder 1988; Hildrew & Ormerod 1995; Kowalik *et al.* 2007). Not all taxa were equally affected by acidification, since the acid tolerance of each individual species varies (e.g. Hämäläinen & Huttunen 1996). For instance, many fish species are vulnerable to toxic effects, often through the effect of aluminium on gill function, while reproduction is commonly disrupted (Jensen & Snekvik 1972). As a result, fish are largely constrained to streams with a pH

over c 5.5 (Schindler 1988), whereas acid-tolerant stoneflies such as leuctrids and nemourids can be found in strongly-acidified systems (e.g. Hildrew & Ormerod 1995; Hildrew 2009).

In addition to the direct toxicological effects on acid sensitive taxa, there are also indirect effects on freshwater ecosystems, mediated by food web interactions and/or disruption of key ecosystem processes. For instance, microbial decomposition of basal resources can be reduced in low pH systems (Hildrew *et al.* 1984; Dangles *et al.* 2004; Pye *et al.* 2012), as well as primary and bacterial production (Mulholland *et al.* 1986, 1992), which could in turn limit production by species higher in the food web. At the top of the food web, however, the loss of acid-sensitive predators such as fish can lead to the indirect, 'top-down', release from predation of more acid-tolerant, invertebrate 'meso-predators' (Hildrew 1992). The combination of these direct and indirect effects make the question of the effects of acidification on aquatic communities a complex one, with community structure and ecosystem processes profoundly affected. What was clear from many large-scale studies is that the overall diversity of the community is usually higher in circumneutral rather than these acidified systems (Driscoll *et al.* 2001; Layer *et al.* 2010b; Lacoul *et al.* 2011; UKAWMN 2010).

To prevent further damage to aquatic ecosystems, it was clear that a decrease in emissions of oxides of sulphur (SOx) would be needed. Industrialised nations were implicated in acidification as a result of such emissions. Various legislative restrictions on industry were introduced, which include the Gothenburg Protocol of 1999 (RoTAP 2012; Traister *et al.* 2013), the Convention on Long-Range Transboundary Air Pollution of 1979 (Rose *et al.* 2004), the Acid Rain Program of 1995 (Pound *et al.* 2013) and the East Canada Acid Rain Program (Stoddard *et al.* 1999). Legally-binding conventions such as these led in turn to declines in emissions, either via a decrease in fossil fuel combustion or through the introduction of chemical scrubbers to reduce SOx in emissions (Monteith & Evans 2001). As a result, the chemical recovery of freshwaters is now widespread (e.g. Battarbee *et al.* 1988; Stoddard *et al.* 1999; Skjelkvåle *et al.* 2005; Fowler *et al.*

2005), although biological recovery is much less clear (Yan *et al.* 2003; Kowalik *et al.* 2007; UKAWMN 2010). The lessons learned from European industrial pollution are especially relevant when viewed in the context of massive industrialisation and vast emission increases in developing nations, such as China, India, Brazil and Vietnam (e.g. Seip *et al.* 1999; Alewell *et al.* 2000; Guo *et al.* 2010), which are already facing similar ecological problems in the 21st century to those that North America and Europe observed in the 20th century. In addition, other climatic pressures on freshwater systems are becoming increasingly apparent, such as eutrophication, climate change and drought, and will most likely alter/confound the trajectory of recovery observed to date (Battarbee *et al.* 2013). Some of these stressors, such as increasing nitrogen load, can have strong interactive effects with pH (Simon *et al.* 2010), which could further confound existing predictions and models (e.g. UKAWMN 2010; RoTAP 2012).

1.2 - Chemical and biological recovery from acidification

Governmental legislation to lower acid deposition proved effective, with SO_4^{2-} concentrations in rainwater reduced by 63% in the USA from 1985 to 1996 (Stoddard *et al.* 1999) and SO_2 deposition in the UK reduced by 71% from 1986 to 2001 (Fowler *et al.* 2005). To establish if such emission controls were having a tangible effect on previously acidified systems, therefore, it was necessary to monitor vulnerable sites both chemically and biologically (Battarbee *et al.* 2013). Monitoring schemes were therefore set-up; one such long-term programme was the UK Acid Waters Monitoring Network (UKAWMN: http://awmn.defra.gov.uk), which consisted of 11 streams and 11 lakes throughout the United Kingdom, in areas on acid-sensitive geology.

Sampling took place (and at most sites continues to take place under the recently rebadged UK Upland Waters Monitoring Network) at regular intervals on an annual basis, with an integration of both biological (e.g. species richness) and chemical (e.g. pH) indicators. Schemes such as this eventually revealed the widespread chemical recovery of many of the sites, in terms of rising pH and acid neutralising capacity, and declining non-marine sulphate (xSO_4) and labile aluminium concentrations (e.g. Fowler *et al.* 2005; Davies *et al.* 2005; Kowalik & Ormerod 2006). While this chemical recovery is not complete - heavy metals concentrations remaining largely unchanged at some sites (Rose *et al.* 2004) - it is pronounced at a large proportion of them (UKAWMN 2010; Monteith *et al.* 2013).

However, this has not yet translated into a corresponding biological recovery (Monteith *et al.* 2005; Layer *et al.* 2010b). While there have been isolated instances of biological recovery in acidified fresh waters (Woodward & Hildrew 2001; Tipping *et al.* 2002; Hildrew 2009), it has generally been patchy and limited (Battarbee *et al.* 2013; Murphy *et al.* 2013). It is not fully understood as to why this may be, although various hypotheses have been put forward, none of which are necessarily mutually exclusive.

It has first been suggested that the persistent recurrence of acid-sensitive assemblages is inhibited by reoccurring acid episodes, which may still occur when acidifying pollutants from emissions (which have been accumulated on winter snow drifts, or may still be stored in catchments), are released in runoff following spring melts or rainstorms (Lepori *et al.* 2003; Ormerod & Durance 2008). This in turn markedly reduces pH and increases toxic metal concentrations (Kowalik *et al.* 2007). These intermittent decreases in pH are suggested to exclude acid-sensitive species, despite a long-term overall increase in chronic acidity and mean pH. However, acid episodes are more pronounced in streams than in lakes, but these show no significant differences in biological recovery, as would be expected if acid episodes were solely responsible (Hildrew 2009; Murphy *et al.* 2013).

Another hypothesis regarding potential limitations on recolonisation by acid-sensitive taxa following chemical recovery was thought to be dispersal limitations, originating from a potential lack of non-acidified refugia within range of the recovering system (e.g. Rundle *et al.* 1995; Bradley & Ormerod 2002; Petersen *et al.* 2004) – a problem arising from the naturally fragmented nature of freshwater systems, especially headwater streams (Hildrew 2009; Hagen *et al.* 2012). This was thought to be especially pronounced given that most headwaters, which act as refugia for stream invertebrates, were heavily vulnerable to acidification (Hildrew 2009). However, indirect genetic evidence shows that invertebrate genetic diversity is often not markedly different between headwaters of different catchments with no direct aquatic connection (e.g. Wilcock *et al.* 2007; Didham *et al.* 2012). This suggests that there is significant dispersal capacity, and that dispersal across watersheds is possible and/or frequent. Empirical studies of dispersal (Raddum & Fjellheim 2003; MacNeale *et al.* 2005; Masters *et al.* 2007; Lancaster *et al.* 2010) provide extensive evidence of widespread inter-catchment dispersal, at least for aquatic insects, between headwaters in previously acidified catchments. It is therefore unlikely that dispersal limitations are constraining the biological recovery of aquatic insects (Yan *et al.* 2003).

Ecological 'resistance' to recovery has also been suggested as a reason for non-recovery; the hypothesis is that community structure and ecosystem processes in acidified systems prevent recolonisation by acid-sensitive species, causing lags, or hystereses, in biological recovery via some process of 'internal inertia'. This effectively results in acid systems being more resistant to structural change than circumneutral ones (Layer *et al.* 2010b). For example, many algal 'grazers' are often also acid-sensitive (e.g. snails and many mayflies) whereas some detritivorous species, which act as functional 'shredders', are also often acid-tolerant (Dobson & Hildrew 1992; Ledger & Hildrew 2005). However, such feeding guilds do not necessarily imply a fixed diet, but rather that there is a degree of flexibility and opportunism (e.g. Lundberg *et al.* 2000). Thus, some acid-tolerant purportedly detritivorous species seem to expand into grazing niches if acid-sensitive

species are lost (Ledger & Hildrew 2000a, b). Ledger & Hildrew (2005), who demonstrated that the ecosystem process of herbivory in acid streams is maintained by generalist acid-tolerant species, suggested that flexibility in acid-tolerant species in switching to algal food, might retard or even exclude the recolonisation of more acid-sensitive grazers.

If this last hypothesis is correct, then it suggests that the hitherto modest recovery from acidification has complex ecological, as well as more obvious toxicological, explanations. What is clear is that reversal of chemical pollution has not resulted in a simple return to more 'pristine' biological conditions. Shifts in community structure and ecosystem processes could be responsible for this, and thus and an understanding species interactions, including networks of interactions, is important (Ings *et al.* 2009; Woodward *et al.* 2013). The study of ecological networks allows analysis of the biological assemblage as a whole, without focussing purely on individual species and/or feeding guilds. Food webs, in particular, have become a well-studied and effective focus for studying the response of a community undergoing environmental stress (Oleson *et al.* 2010; Ledger *et al.* 2012; O'Gorman *et al.* 2012; Thompson *et al.* 2012; Clitherow *et al.* 2013).

1.3 - Food webs: Function, stability and structure

Food web research has been ongoing since the late 19th century, when Darwin (1859) made his famous reference to 'the tangled bank', followed by Camerano (1880) and Forbes (1887) publishing the first recognisable food webs. Early works in the field (e.g. Elton 1927; MacArthur 1955) argued the complexity observed in natural ecological networks, such as food webs, is key to maintaining stability – by enabling systems to withstand perturbations, such as species invasion or increased environmental stress (e.g. acidification). However, the classic modelling work of May (1972, 1973), based on experimental microcosms, argued the opposite conclusion; that simple systems are inherently more stable and that the complexity observed in natural systems must arise from unknown factors; which he dubbed ' devious strategies'. Using these early forms of dynamic modelling, it was demonstrated that many of the complex trophic interactions that are observed in natural systems should lead to lower levels of stability (Pimm 1980). An early problem for food web ecologists – a problem that remains to this day (albeit to a lesser extent) – is that there are relatively few well resolved and quantified food webs that have been described (but see Cohen *et al.* 2003; Woodward *et al.* 2005a). However, the study of ecological networks, and specifically food webs (due to the relatively ease of measuring trophic links between species compared to the more subtle links observed in other forms of ecological network, such as host-parasatoid and mutualistic), has become increasingly popular within the literature in recent years (Ings *et al*. 2009), with community stability being measured by increasingly sophisticated modelling techniques, complemented by empirical data. However, stability can be a fluid concept. McCann (2000) argues that the concept of network stability is not based on a single definition, but can be split into two broad categories; definitions based on dynamic stability (such as equilibrium stability, which concerns a system's response to perturbation in order to return to an equilibrium stable state) and more static definitions based on the network's ability to withstand change,

primarily those of resilience (i.e. the speed at which a community can recover after either a biotic or abiotic perturbation) and of resistance (i.e. the ability of a community to resist alteration as a result of a perturbation). Early works focussed on species diversity as an indicator of stability (e.g. Tilman & Downing 1994; Tilman *et al.* 1996), although it has become clear that more complex factors have a significant effect on stability, such as body size and interaction strengths (De Ruiter *et al.* 1995; McCann *et al.* 1998; Aufderheide *et al.* 2013). There has also been a move towards considering the underlying drivers of food web structure and processes (e.g. Ings *et al.* 2009); an approach which incorporate the species traits that govern food web stability, rather than the relatively simplistic presence/absence approach originally favoured in the early published literature (e.g. Cohen 1978; Hall & Raffaelli 1993). It is such drivers, and their impact on acidified communities, that are the main focus of this thesis.

Body size is often cited as one of these key traits that govern food webs (e.g. Peters 1983; Warren & Lawton 1987; Woodward *et al.* 2005b; Jacob *et al.* 2011). The role of body size in ecology has been recognised for a long time, with Huxley (1932) one of the first to highlight that a number of dependent variables (e.g. morphological measurements) scale predictably with body mass via a process called allometry. It is only recently, however, that its role has been explored more thoroughly, via studies of allometric scaling relationships (e.g. Cohen *et al.* 2003; Reuman *et al.* 2009) and foraging constraints (Petchey *et al.* 2008). In many cases, it appears that body size is often the primary driver of structure and dynamics (Williams & Martinez 2000; Woodward *et al.* 2005b). Size-structuring is particularly evident within many aquatic food webs (Jennings & Mackinson 2003; Jonssen *et al.* 2005; Woodward *et al.* 2005a; Brose *et al.* 2006a; Layer *et al.* 2011; O'Gorman *et al.* 2012) and, as a result, body size can be used as a single variable in aquatic food web studies to capture a large amount of variation, as ecological traits such as abundance, reproductive rate, dietary width and nutrient turnover are all constrained to some degree by body

size (Brown *et al.* 2004; Woodward *et al.*. 2005b). Of particular significance is the observation that numerical abundance often follows a three-quarters allometric scaling relationship with body mass ($M^{-0.75}$) (Damuth 1981; Brown & Gillooly 2003; Woodward *et al.* 2005b). This mass-abundance relationship, whilst not strictly universal, has been shown to have a strong impact on food web topology (Cohen *et al.* 2003) and community stability (Louielle & Loreau 2004; Otto *et al.* 2007) when viewed from a species-averaged perspective, as opposed to individual-based approaches.

All species are linked by some form of either direct or indirect interactions (Paine 1988). The strength and pattern of interactions between predators and their prey are another of these drivers often cited as a key determinant of system stability (Berlow *et al.* 2004; O'Gorman & Emerson 2009; Montoya *et al.* 2009). The view of May (1972, 1973) that stability is intrinsically linked to simplicity has since been countered (Polis 1998), with the suggestion that many weak interactions, paired with few strong interactions, can create a complexity that allows stability (McCann *et al.* 1998; Montoya *et al.* 2009). Both experimental data and food web theory have demonstrated 'real-world' food webs characterised by this pattern (Paine 1992; Fagan & Hurd 1994; Neutel *et al.* 2002), and it is thought that higher proportions of weaker links generate negative covariances, which can dampen the destabilising potential of any perturbation in the system (Berlow 1999; Williams *et al.* 2002; O'Gorman & Emmerson 2009; O'Gorman *et al.* 2010). Consequently, it is likely that the loss of weakly-connected species could be damaging for communities (Christianou & Ebenman 2005). A key form of interaction is that of the functional response, which describes the change in predator consumption of prey (i.e. intake rate) when density varies (i.e. availability). There are generally accepted to be three types of functional response, as defined by Holling (1959) (see Figure 1). Type I responses are the simplest, and assume a linear increase in consumption as prey availability increases, with little concern for searching for prey or handling time. Type II responses are generally seen as the most common in natural systems, and

demonstrate a decrease in consumption rate as density increases, as a result of searching/handling time increases, up to an asymptote when prey numbers saturate the ability of predators to consume them. These responses have been described as destabilising in natural systems (Aljetlawai *et al.* 2004; Brose *et al.* 2006b). Type III responses are similar to Type II, in that they also exhibit an asymptote at high prey densities, but differ in that at low densities there is lag followed by a density-dependent increase in consumption. These responses are rarer in natural systems, but are widely believed to have a stabilising effect on communities. However, while trophic direct links such as these are clearly important in maintaining food web structure and function, it is becoming apparent that other, more subtle indirect effects are also vital (Ings *et al.* 2009).

The role of indirect interactions (i.e. when a direct link between two species is mediated by another) in governing ecological networks has begun to be recognised in recent decades (Beckerman *et al.* 1997; Schmitz *et al.* 1997; Luttberg & Kerby 2005; Petchey *et al.* 2008), The emergent effects of behavioural or trait-mediated responses can have powerful impacts on ecosystems (Woodward 2009). For example, the defence mechanisms often displayed by prey species in the presence of a predator are well-documented, with responses such as active avoidance and activity reduction by prey apparent (e.g. Sih 1993; Sih *et al.* 1998; Priesser *et al.* 2005). By incorporating this behavioural information into direct food web construction, a more complete understanding of community dynamics is possible. Work into predator foraging theory (Beckerman *et al.* 2006; Petchey *et al.* 2008) has demonstrated this by using diet breadth models to model network connectance; an example of emergent network properties based on individual behaviour. In addition, trait-mediated indirect interactions can play a vital role in food web complexity, as they have the ability to stabilise a system (McCann *et al.* 1998), but the nature of this role can be highly complex (Montoya *et al.* 2009). These indirect interactions, such as apparent competition, are seemingly widespread in aquatic ecosystems (Mither & Lawton 1986;

Huang & Sih 1990), in which multiple direct and indirect effects can occur simultaneously (Knight *et al.* 2005; Montoya *et al.* 2009).

It is therefore apparent that the factors governing the inherent stability a community, and the ecological network that it forms, are complex. In order to understand how stability persists in natural systems faced with environmental stress, such as acidification, a whole-system approach is needed, from bottom-up energetic inputs to top-down pressures from apex keystone species. The long-term scientific study of Broadstone Stream, and the surrounding streams of the Ashdown Forest, provides several ideal sites for aquatic research into these areas.

1.4 – Broadstone Stream and the Ashdown Forest

Broadstone Stream and its food web is one of the best-studied ecological networks in the world (Hildrew 2009). It has been the subject of extensive research from the 1970s to present (e.g. Townsend & Hildrew 1976; Hildrew *et al.* 1985; Lancaster *et al.* 1991; Woodward & Hildrew 2001, Schmid-Araya *et al.* 2002; Larrañaga *et al.* 2010; Layer *et al.* 2011) with a fully-resolved but relatively species-poor food web that nonetheless is strongly size-structured and reticulate, with over 600 interactions documented (Woodward *et al.* 2005a, 2010a). The long-term study of this site has allowed the observation of chemical recovery– annual average pH has risen from approximately 5 in the 1970s to 5.6 in the 2000s (Layer *et al.* 2011). Alongside this has been noticeable biological recovery, entailing subsequent changes on food web structure (Woodward *et al* 2005a; Layer *et al.* 2011). In the 1970s and 1980s, the predator guild was dominated by the large invertebrate predators *Plectrocnemia conspersa* Curtis, a caseless caddisfly, and *Sialis fuliginosa* Pictet, an alderfly, and the food web was relatively stable, with few novel species

colonising, despite amelioration of acidification (Hildrew *et al.* 1985). However, the relative abundances of the resident taxa were subject to change (Townsend *et al.* 1983), with chemically recovery eventually allowing the irruption of a third invertebrate top predator (*Cordulegaster boltonii* Donovan), a large dragonfly nymph, whose population increased radically and rather abruptly from <0.1 individual m^{-2} in the early 1970s to the late 1980s to 70 individuals m^{-2} in the mid-1990s (Woodward & Hildrew 2001) in what was effectively an invasion, as if of a new species. This had consequences for the dynamic stability of the food web, with complexity and food chain length both increasing – both have destabilising implications for the network (Woodward *et al.* 2005a). As acidity has continued to ameliorate, an acid-sensitive vertebrate predator (*Salmo trutta* L.), has been recorded (Layer *et al.* 2011), invading this previously exclusively-invertebrate network (*S. trutta* can only maintain viable populations at pH approx. 5.4 and higher (Jensen & Snekvik 1972)). It is important to note here that the term 'invader', when applied to both *C.boltonii* and *S.trutta*, does not imply that they are non-native species; rather that they are new members of the food web that have invaded locally from a larger regional species pool. It is also likely, although it can only be hypothesised because the data do not extend further back than the early 1970s, that both were present in the stream prior to the onset of anthropogenic acidification in the 19^{th} and 20^{th} centuries, which makes them particularly relevant in the context of biological recovery.

Aquatic systems in general, and Broadstone Stream in particular, have been shown to contain predators in which the diet of a 'mesopredator' is a nested subset of larger predators i.e. as size increases so does breadth of diet (Woodward & Hildrew 2001; Woodward & Warren 2007; Yvon-Durocher *et al.* 2008). There are also size-mediated feeding patterns, such as asymmetric intraguild predation, mutual predation and cannibalism (Woodward & Hildrew 2002b). The impact of body size is of particular relevance here, as *C.boltonii* is an order of magnitude larger than the next smaller invertebrate in the system (*Sialis fuliginosa*), and *S.trutta* is several orders of

magnitude larger again (Layer *et al.* 2011). The presence of *S.trutta* in a previously invertebrate-dominated system has the potential to restructure the entire food web. The structure and stability of systems can be altered profoundly by the invasion of a novel predator, which can represent a perturbation as severe as an alteration of environmental conditions (e.g. acidification). Species invasion can result in impacts such as species extinction (Townsend 1996; Simon & Townsend 2003) and trophic cascades (Flecker & Townsend 1994; Knight *et al.* 2005) i.e. amplified effects of species change across trophic levels. If the invader is a new apex predator, such as brown trout in Broadstone, the impact may be extensive, with new feeding links created and existing interactions being shifted (McPeek 1998). The established community in Broadstone, however, might be relatively resistant to invasion, as suggested by several properties of its food web. *Salmo trutta*, which was first detected in the system in 2005 (Layer 2010), is only the second successful invader by a novel predator since the beginning of long-term study began; a period spanning four decades. Indeed, the first successful predator invasion, that of *C.boltonii*, increased web complexity and connectance (Woodward & Hildrew 2001), but no local extinctions of its prey or potential competitors have thus far been observed (Woodward *et al.* 2005a). However, *S.trutta* is several orders of magnitude larger and considerably more mobile than *C.boltonii*. This marked increase in size suggests that brown trout will be the new apex predator within the food web, thus increasing food chain length, altering energy fluxes and patterns of interaction strength (Layer *et al.* 2011). As a result, the impact of direct top-down apex predation on the food web certainly has the potential to alter the stability of the system, but the behavioural consequences are less well understood. If the activity of prey and/or the attack rate of the intermediate (meso-) predators are altered in the presence of a new apex predator, such as trout, it can alter the existing dynamics of a network (Sih *et al.* 1998).

1.5 – Aims and thesis structure

The main aim of this project was to examine the impacts of amelioration of acidity in freshwater ecosystems, across levels of organisation from individuals to the ecosystem, and to assess why the pace of biological recovery, from the acidifying events of the 20th century, in such systems is lagging behind widespread and noticeable chemical recovery. To do this, I combined data from both laboratory and field experiments with those from a long-term survey of the Broadstone Stream 'model' food web, in order to test various hypotheses relating to biological inertia and stability in stream communities. Chapters Two to Four contain analysis of Broadstone Stream, which has a highly-resolved and reticulate food web resulting from decades of intensive/extensive study. Chapter Five expands the scope to numerous other interconnected streams over spatial, temporal and chemical gradients. Figure 2 shows which specific links/interactions within the food web are investigated in each data chapter.

In Chapter Two, "Simulating species interactions in recovering communities: functional responses of acid-tolerant predators to prey characteristic of acidic and circumneutral streams", I explore individual predator-prey interactions between the resident Broadstone macroinvertebrate predatory guild and a range of acid-tolerant and acid-sensitive species, across a range of sizes and densities, hypothesising that these interactions, and the resulting functional response, could confer stability at lower levels of organisation (individual and population) in acid communities which are potentially subject to invasion by acid-sensitive species as chemical recovery continues.

In Chapter Three, "Simulating indirect interactions between acid-tolerant and acid-sensitive species", I expand this avenue of investigation into a basic network-level approach (one predator species, two prey species) by focussing on indirect interactions, those of apparent competition and behavioural responses, rather than direct antagonistic interactions. I hypothesise that there could

be inherent resistance in acid communities to biological recovery at a higher level of organisation as a result of these indirect factors, which could potentially inhibit colonisation by acid-sensitive taxa.

In Chapter Four, "Effects of long-term amelioration of acidification and invasion by a vertebrate predator on a well-characterised food web", I look again at higher level of organisation by examining the entire network of Broadstone Stream, and examined how it has responded to long-term changes in physicochemical conditions over several decades, as well as experimentally testing the potentially destabilising effects on the whole food web of the brown trout invasion. I hypothesise that the resulting magnitude of increases in interaction strength could alter the structure and function of the system, leading to directly induced instability in the network.

I then expand the scope of the thesis beyond a single system in Chapter Five ("Long-term amelioration of acidity accelerates decomposition in headwater streams") to examine changes in decomposition, a key ecosystem-level process, across a pH gradient in 31 streams in the Ashdown Forest, SE England. By comparing these data to a similar study conducted in the 1970s, I was able to observe decadal-scale responses, at an ecosystem-level, in the amelioration of acidity by measuring shifts in the rate of decomposition, and discuss the wider ecological implications for the communities of these streams, speculatively relating this ecosystem process to changes in the structure of the food web.

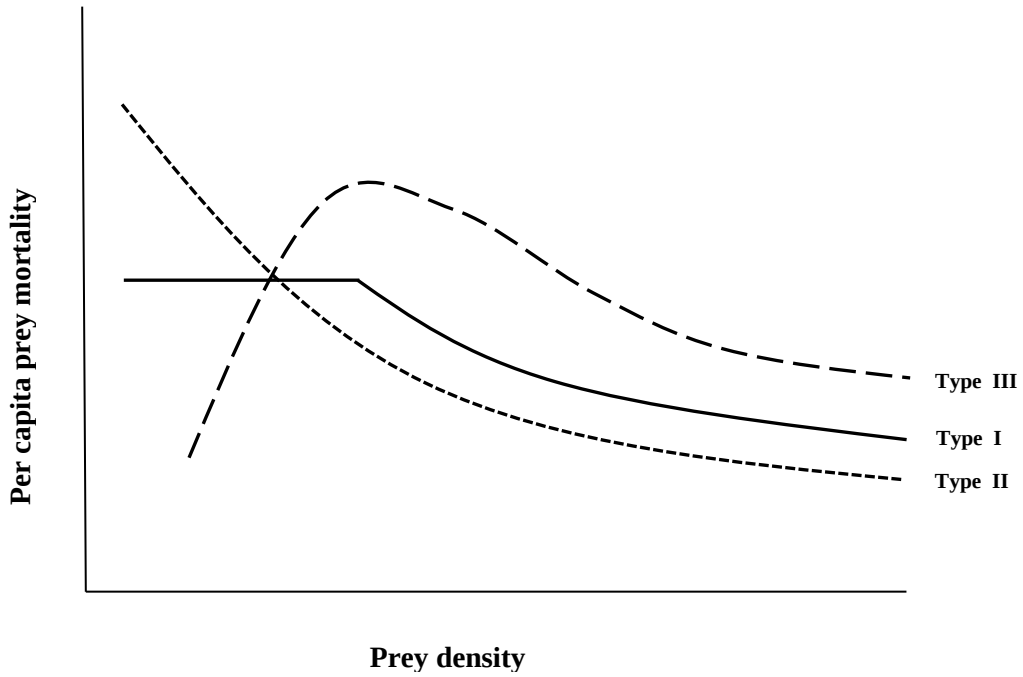

Figure1. Theoretical functional per capita prey mortality curves for Type I, II and III functional responses. Note the density-dependence of Type III.

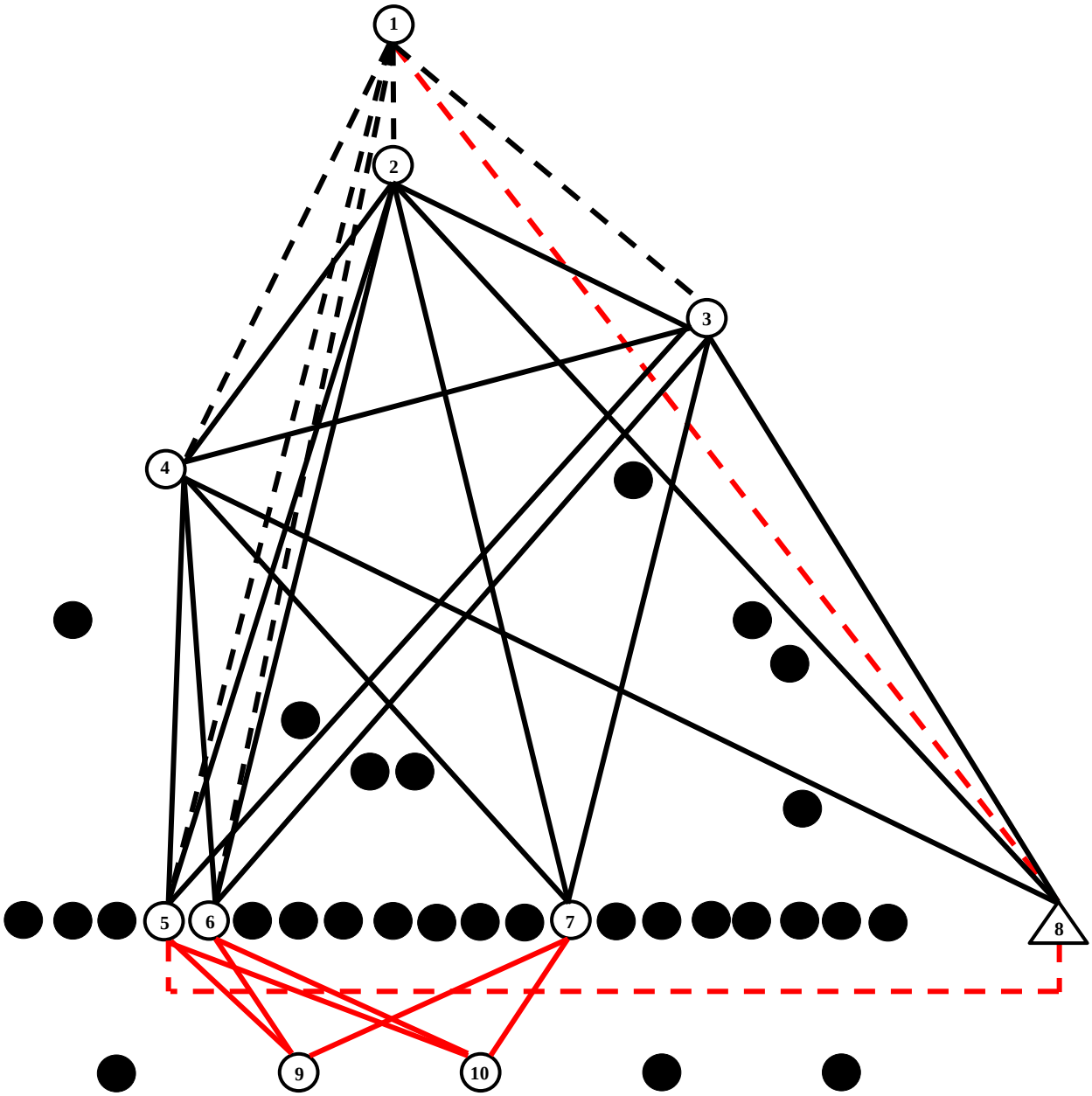

Figure 2. Schematic connectance food web of Broadstone Stream, with links coded to specific chapters of this thesis. Key to symbols: Open circles - experimental resident taxa, closed circles - other resident taxa, open triangle - a simulated invasive taxon. Black continuous links (Chapter Two), – direct trophic interactions, red dotted links (Chapter Three) and black dotted links (Chapter Four) - indirect trait-mediated interactions, red continuous links (Chapter Five) – detrital input. Key to species: 1 – *Salmo trutta*, 2 – *Cordulegaster boltonii*, 3 – *Sialis fuliginosa*, 4 – *Plectrocnemia conspersa*, 5 – *Nemurella pictetii*, 6 – *Leuctra nigra*, 7 – *Paraleptophlebia submarginata*, 8 – *Baetis rhodani*, 9/10 – Organic detritus. Redrawn from Woodward *et al.* 2005a and Layer *et al.* 2011.

Chapter Two: Simulating species interactions in recovering communities: functional responses of acid-tolerant predators to prey characteristic of acidic and circumneutral streams.

Abstract

The chemical recovery of fresh waters across Europe and North America from anthropogenic acidification has not yet led to a consistent biological recovery. One possible explanation is that interactions between individuals and populations of the acid-tolerant and acid-sensitive species may cause hystereses or lags at higher organisational levels (e.g. the food web). This could result in inertia, whereby the reassembly of an acid-sensitive community does not simply follow the reverse trajectory of the response to acidification as chemical conditions ameliorate. Here, I present the results of experiments on predator-prey interactions, using combinations of stream invertebrates with differing pH optima and across a range of prey densities and temperatures. I fitted a variety of functional response models to these data, hypothesising that Type III functional responses were widespread, inferring a density-dependent stabilising effect of predators on prey at low prey density. The largest of the acid-tolerant predators used (nymphs of the dragonfly, *Cordulegaster boltonii*) caused high prey mortality, most particularly on acid-sensitive mayflies. The large invertebrate apex predators of acidified streams might delay the re-establishment of an acid-sensitive fauna, despite the amelioration of water chemistry, due to the 'damping' of population fluctuations of acid-sensitive prey. The population-level manifestation of these interactions could potentially increase the resistance of acidified systems, which are already

known to be structurally stable at the network level. Stability conferred at these different levels of organisation could be one aspect of the apparent resistance to structural change of the webs of acid streams, in the face of chemical change, and offer a further potential explanation for the lack of large-scale biological recovery of acidified streams.

Introduction

The widespread loss of biodiversity in acidified waters across large areas of Europe and North America, and the diagnosis of anthropogenic acidification as the main cause (e.g. Flower & Battarbee 1983) led to international agreements and subsequent legislation to reduce acidifying emissions, such as the Convention on Long-range Transboundary Air Pollution of 1979 (Hettelingh *et al.* 2008) and the Gothenburg Protocol of 1999 (RoTAP 2012). As a consequence, large-scale chemical recovery of acidified freshwaters in the UK began to be detected through long-term monitoring in the 1980s (Monteith & Evans 2000; UKAWMN 2010; RoTAP 2012). However, this has not been accompanied by a corresponding widespread biological recovery, such as the expected reassembly of an acid-sensitive fauna (Ormerod & Durance 2009). There are three main hypotheses, which are not necessarily mutually exclusive, as to why this might be: occasional acid episodes at high flows (sufficient to prevent re-establishment of acid sensitive species), dispersal limitations on acid-sensitive species (slowing their recolonisation of newly suitable habitats) newly and 'ecological inertia' (including possible interactions between the incumbent acid-tolerant community and acid-sensitive species, which inhibit community change).

The first hypothesis of persistent acid episodes suggests that, despite a general upwards trend in mean pH, acid events still occur due to spikes in acidified run-off after snow melt or storms

(Kowalik *et al.* 2007; Feeley *et al.* 2013) which are sufficient to prevent the persistence of acid-sensitive species. This should apply more strongly to streams than to lakes, as the chemistry of the latter is much less episodic and more buffered by the larger volumes of water involved (Hildrew 2009; RoTAP 2012), although as yet ecological recovery certainly seems no stronger in lakes than in streams (Murphy *et al.* 2013). The second concerns limitations on dispersal, with the naturally fragmented nature of fresh waters (Woodward & Hildrew 2002a; Hildrew 2009; Hagen *et al.* 2012) potentially preventing re-colonisation from adjacent less acid systems (Bradley & Ormerod 2002; Petersen *et al*. 2004). However, limited invertebrate genetic diversity have been observed between catchments (Wilcock *et al.* 2007; Didham *et al.* 2012), suggesting long-distance adult dispersal is possible, and that only a few individuals are needed to repopulate a system. The high dispersal capacity of some aquatic invertebrates between relatively distant headwaters has been demonstrated (Raddum & Fjellheim 2003; MacNeale *et al.* 2005; Masters *et al.* 2007; Lancaster *et al.* 2010), as has strong density-dependent mortality early in the life-cycle in large-scale population manipulations (Hildrew *et al.* 2004). This suggests that dispersal constraints are not a ubiquitous "bottleneck to ecological recovery" (Yan *et al.* 2003); at least, not in the case of aquatic insects. A third conjecture is there is an 'internal inertia' (Ledger & Hildrew 2005; Monteith *et al.* 2005; Woodward 2009) caused by the intrinsic stability of acidified food webs, which appear to be more resilient and resistant to changes in structure than circumneutral ones (Townsend *et al.* 1987; Layer *et al.* 2010b; Layer *et al.* 2013). This stability is manifested via community characteristics such as increased robustness (Layer *et al.* 2010b), trophic generalism (Ledger & Hildrew 2005; Layer *et al.* 2013) and persistence (Townsend *et al.* 1987; Woodward *et al.* 2002).

However, the lower levels of organisation could also confer stability, in addition to these higher-level food web effects, simply via density-dependent population regulation by predation and/or competition for resources or enemy-free space in smaller two or three-species modules within the

food (e.g. Speirs *et al.* 2000; Hildrew *et al.* 2004). One aspect of species interactions within acidified ecosystems are those arising from competition for resources. Acid-sensitive species are predominantly specialist grazers, whereas acid-tolerant species are generalist herbivore-detritivores (Ledger & Hildrew 2005; Layer *et al.* 2013). This trait contributes to their success in acid streams, as the basal resources are detrital dominated. However, acid-tolerant species are also able to exploit the few algal resource available in acid systems. This means that, as chemical conditions recover, the large populations of herbivore-detritivores can inhibit re-establishment of specialist grazers (Ledger & Hildrew 2005), thereby increasing the resistance of the system. Another aspect of the role of species interactions in inhibiting straightforward biological recovery is that the numerous acid-tolerant invertebrate predators could prevent re-colonisation by acid-sensitive taxa, via strong predator-prey antagonistic interactions leading to top-down control of prey population densities (e.g. Layer *et al.* 2011). While the loss of acid-sensitive taxa occurs once pH tolerance falls below a certain threshold (Hämäläinen & Huttunen 1996; Hildrew 2009; Layer *et al.* 2013), it is less clear how species interactions relate to these species losses, and how the food web reassembles following chemical recovery.

The trophic response of individual predators to changes in prey density is a key component of predator-prey interactions and, ultimately, of food web dynamics (Aljetlawi *et al.* 2004; Sarnelle & Wilson 2008; Sentis *et al.* 2013), and these can be measured via functional response models. There has been substantial research into predator functional responses, which can be defined as quantitative models of interaction strength (e.g. Holling 1959; Rogers 1972; Hildrew & Townsend 1977; Jescke *et al.* 2004; Rall *et al.* 2011), and have been divided into three broad types (Type I, Type II & Type III; see Holling 1959). Of particular relevance in the context of stability are Type III responses, which have mostly been experimentally demonstrated in *Daphnia* species in aquatic systems (Scheffer & De Boer 1995, Jeschke 2004, Sarnelle & Wilson 2008) or benthic isopods (Aljetlawai *et al.* 2004). The key characteristic of this response type is that it can, lower values of

prey density, have a stabilising effect on predator-prey dynamics (Aljetlawai *et al.* 2004), and reduce fluctuations in prey populations (Murdoch & Stewart-Oaten 1975). The density-dependent Type III response takes a sigmoidal form, in which the number of prey taken by predation, in relation to prey density, is described by an initial lag due to limitation by encounter rate, followed by an upward sweeping curve (where prey mortality is density dependent), before reaching an asymptote at which predation rate becomes saturated and handling constraints are apparent. At this saturated point there is the potential for destabilising effects to arise as predators are no longer able to control prey populations. Accordingly, stability (or lack thereof) of a prey population in a system could be partially governed by the prevalence of Type III responses.

Broadstone Stream (an acidified system in southern England and subject of ecological research since the early 1970s) is one such system, and one of the best-described food webs published to date (Hildrew *et al.* 1985; Woodward *et al.* 2005a; Hildrew 2009; Oleson *et al.* 2010; Layer *et al.* 2011). It is an ideal system for studying long-term change in response to deacidification, as mean annual pH has risen from 5 in the 1970s to 6.2 in response to decreased anthropogenic acidifying emissions. Broadstone Stream is connected to several circumneutral streams in the same catchment, as well as having the circumneutral waters in its own lower reaches (Ledger & Hildrew 2001), meaning that re-colonisation of acid-sensitive species is not dispersal limited (Petersen *et al.* 2004; Masters *et al.* 2007). While there is a small waterfall present towards the lower reach (which may have presented a physical barrier to in-stream dispersal), it has not prevented the colonisation of brown trout (Layer *et al.* 2011), and re-colonisation by winged insects is possible across the wider catchment (Petersen *et al.* 2004; MacNeale *et al.* 2005; Masters *et al.* 2007). It would therefore seem reasonable to expect that a more acid-sensitive assemblage would have established within the stream, but this is not the case. Rather, the community has seen several irruptions and/or invasions of progressively larger top predators as chemical conditions have improved (Woodward & Hildrew 2001; Hildrew 2009; Layer *et al.*

2011), highlighting that recovery is not simply a reversal of the response to acidification, as well as suggesting top-down control of the prey assemblage via direct consumption or apparent competition by generalist predators could be creating inertia in the food web.

An early study within Broadstone Stream (Hildrew & Townsend 1977) identified that the large invertebrate predator *Plectrocnemia conspersa* Curtis aggregated on patches of high prey density, which amplified its impact in these areas, while relieving pressure on areas of low density (Hildrew & Townsend 1982), which could in turn stabilise predator-prey dynamics (Begon *et al.* 2006). Further laboratory and field studies into the invasion of a new top predator in the 1990s, the large dragonfly *Cordulegaster boltonii* Donovan, also suggested a strong density-dependent response to prey abundance (Woodward & Hildrew 2002a,c). These experiments were performed in the context of a single predator and no physicochemical change – returning to species interaction experiments using this system, but in the light of significant decadal-scale shifts in chemical conditions within the catchment (Hildrew 2009, Layer *et al.* 2011; Jenkins *et al.* 2013), could help to explain the biological inertia of certain acid-sensitive prey populations. In addition, given the fluxes in population densities of resident species throughout an annual cycle (e.g. peaks in recruitment in the warmer summer months), an understanding of how predator-prey interactions change in response to temperature is crucial in examining if any chemically-mediated patterns are consistent throughout the year. It would be expected that the higher metabolic demands associated with increased temperature (e.g. Brown *et al.* 2004) would lead to correspondingly higher levels of predation.

Consequently, in this Chapter and the next, I extend previous work (Hildrew & Townsend 1977, 1982; Woodward & Hildrew 2002a,c) on (in this Chapter) pairwise predator-prey interactions in Broadstone system, by expanding the suite of both predator and prey species used, in order to ask whether the interactions between the established invertebrate predator guild and a range of acid-tolerant and acid-sensitive prey (pairwise interactions in this Chapter, with three species

interactions in Chapter Three) might be responsible for the inertia of biological recovery in this acid stream, and examine whether the resulting responses explain why the system appears resistant to invasion by acid-sensitive prey species.

To do this, I used laboratory experiments to simulate novel predator-prey combinations that may arise as a result of chemical recovery from acidification. Three species of acid-tolerant, large-bodied invertebrate predators common in Broadstone Stream were exposed to various densities of prey; the latter including both acid-tolerant and acid-sensitive potential colonists common in the area. Previous experiments have focussed only on resident, relatively sedentary, acid-tolerant prey species, such as stoneflies (Hildrew 2009). By staging predator-prey interactions between large-bodied, acid-tolerant predators and more mobile acid-sensitive prey, such as mayflies (Peckarsky 1996), it could be tested whether encounter rate would be higher and consequently mortality rate increased. This, in combination with three-species trials in Chapter Three**,** could offer another explanation as to why acid-sensitive species are unable to invade the otherwise now chemically optimal Broadstone system, which would have implications for other recovering acidified systems experiencing similar lags in biological recovery (e.g. Monteith *et al.* 2005; Layer *et al.* 2010a; UKAWMN 2010).

Consequently, I hypothesised that:

(a) Type III functional responses would be prevalent in the macroinvertebrate predatory guild, which has the potential to exert a stabilising effect on species interactions at the population level. Among common factors accounting for such Type III responses are physical refugia, which might shelter a proportion of prey but are in increasingly in short supply as prey density increases (demonstrated for some pairwise interactions in the Broadstone assemblage by Hildrew & Townsend (1977)).

(b) Acid-sensitive species would be more vulnerable than acid-tolerant species, due to biological traits such as higher mobility increasing the encounter rate with acid-tolerant predators, which leads in turn to higher mortality through higher attack rates. The consequences for the assemblage composition and recovery of acid-sensitive species are then tested in three-species trials in Chapter Three.

(c) The magnitude of any changes in mortality would increase as temperature increased, due to increased metabolic demand of both predators and prey.

Methods

Study sites

Broadstone Stream (51°05'N 0°02'E) is a spring-fed headwater of the River Medway in SE England (see Chapter One for further details), which was very acidic (mean pH c. 5.0) when first studied in the early 1970s, but it has seen a substantial amelioration in pH since then, particularly so in the summer (Woodward *et al.* 2002). This long-term rise in pH, from 5 to 6.2 (Hildrew 2009; Layer *et al.* 2011), is consistent among formerly much more acidic streams in the surrounding Ashdown Forest (Hildrew 2009; Jenkins *et al.* 2013). The macroinvertebrate benthos of Broadstone remains relatively species-poor compared to base-rich systems (Layer *et al.* 2010b) and dominated by a core assemblage of 24 acid-tolerant macroinvertebrates, with abundant insect predators, the largest of which are the larvae of the golden-ringed dragonfly *C.boltonii*, which was formerly very scarce but irrupted in the 1990s (Woodward & Hildrew 2001). The two other dominant large-bodied predators are the larvae of the alderfly *Sialis fuliginosa* Pictet, which was

particularly abundant in the 1980s, and the net-spinning caddis *P.conspersa*, which was the dominant predator when the system was first studied in the 1970s (Hildrew & Townsend 1977; Townsend *et al.* 1983).

An acid-sensitive prey species (*Baetis rhodani* Pictet) was collected from a neighbouring site in the Ashdown Forest, Withyham Stream (51°05'N 0°08'E), which is a circumneutral (mean pH c. 7.1) spring-fed headwater and, like Broadstone, a tributary of the River Medway. The macroinvertebrate community of Withyham is dominated by acid-sensitive species, including *Baetis* spp, and has a diverse macroinvertebrate benthos and periphyton (Ledger & Hildrew 2001). A further source of acid-sensitive prey species was the River Cray (51°22'N 0°06'E), a chalk stream in SE England primarily dominated by *Gammarus pulex* L, but with high population densities of *B.rhodani*.

Laboratory experiments

I used the three largest dominant large invertebrate predators from Broadstone (*C.boltonii*, *S.fuliginosa* and *P.conspersa*) as predator species, in combination with four invertebrate prey species that have a range of pH optima (from Hämäläinen & Huttunen 1996; Woodward *et al.* 2002) (see Appendix 1). Three of these species are found in Broadstone, whereas one is not (see Fig 2.1. for conceptual food web): I selected the latter species because (a) it is found in several interconnected local streams (Dobson & Hildrew 1992; Stead *et al.* 2003; Hildrew 2009) and (b) it has been well studied in terms of its ecology, especially in regards to acid streams (Sutcliffe & Carrick 1973; Raddum & Fjellheim 1984; Lepori *et al.* 2003; Tixier *et al.* 2009; Feeley *et al.* 2011). The rationale behind this was to select a likely future invader of Broadstone, should pH continue to rise, as there are populations in nearby streams as well as the lower reaches of

Broadstone itself (see Fig. 2.2 for distribution of predators and prey in the surrounding river network). It has also been found very occasionally in the acidified reach of Broadstone (A.Hildrew, personal communication). I used a pH threshold of c. 5.5 (below which bicarbonate alkalinity and buffering capacity falls to zero and physiological stress increases for many invertebrates and fish (Sutcliffe & Hildrew 1989; Hämäläinen & Huttunen 1996)) to distinguish 'acid-tolerant' species from 'acid-sensitive' species.

I collected all acid-tolerant invertebrate predators and three prey species (*L.nigra, N.pictetii, P.submarginata*) from the acidified reach of Broadstone Stream from October 2011 – March 2013, and the acid-sensitive prey species (*B.rhodani* from Withyham Stream from October 2012 – March 2013. The River Cray was used as an auxiliary source of *B.rhodani* when required. Predator body size was standardised within species by selecting only final instars of *S.fuliginosa* and *P.conspersa* and the largest size-class of *C.boltonii,* as defined by Woodward & Hildrew (2002a) (i.e. instars 12-14).

The experiments were conducted in three litre capacity plastic aquaria ((SAVIC, Belgium), following the protocols of Woodward & Hildrew (2002a). Washed native gravel substratum was added to each aquarium, to a depth of 5cm, with stream water then added to a total depth of 10cm. In previous experiments using these species from Broadstone and Withyham, survival of acid-tolerant species was generally unaffected by transfer to circumneutral conditions (with the exception of *L.nigra*), whereas transfer to acidic conditions caused significant mortality in acid-sensitive species such as *Baetis* sp. & *Gammarus pulex* (Lancaster 1988); accordingly, circumneutral stream water from Withyham Stream was used for all experiments.

Three temperatures (5°C, 10°C, 15°C) were maintained in constant temperature rooms, which replicated the winter, spring and summer mean temperatures in Broadstone, and which were used in previous predator-prey trials (e.g. Woodward & Hildrew 2002a; Layer *et al.* 2011). The light regime was 12 hours light/12 hours dark. Individual predators were first kept separately in the laboratory at 10°C for three days and starved before being added to the test aquaria, to standardise motivational state. They were then introduced to the aquaria before adding prey, to allow individuals 12 hours to adjust to the environment and, particularly, to enable *P.conspersa* larvae sufficient time to spin nets, as this is their primary means of prey capture (Townsend & Hildrew 1979). After 12 hours, prey were added from holding aquaria to begin the experiment. I set initial prey densities at 1, 2, 3, 4, 8, 12 and 16 individuals per arena, but added an additional treatment with 32 individuals for the *C.boltonii*-mayfly experiments, since initial analysis showed no saturation at 16 individuals. This was necessary to describe the asymptote for the feeding curves. The distribution of species in Broadstone and Withyham is naturally patchy (Hildrew & Townsend 1977; Hildrew *et al.* 2004) and in three out of the four prey species, maximum natural patch density exceeded that of the maximum experimental density (see Appendix 1), so the experimental ranges were generally within the natural ranges, although upper densities were above the average for the stream. Each density was replicated four times (see Fig. 2.3 for experimental design). After 24 hours, I removed the predators, then sieved and sorted the gravel in order to count surviving prey. In total, 1008 aquaria were processed (i.e. Three predator species x four prey species x seven prey densities x three temperatures x four replicates).

Statistical analysis

I used the statistical programing language R (R Development Team 2012) loaded with the package "emdbook" (Bolker 2008) after Rall *et al.* (2011) to fit two distinct functional response models to the predator-prey interaction data using the initial prey density vs. number of prey eaten. This was done using Rogers Random Predator equations (RRP) (Rogers 1972), which allow for predator handling time and prey depletion over time (Bolker 2008), as opposed to the functional response models of Hollings (1959), which assume constant prey density.

I used two different functional response equations (a Type II and a Type III) as follows:

- Type II

$$N_e = N_0 - W\left(\frac{(a\ h\ N_0 \exp(-a\ (P\ T - h\ N_0)))}{(a\ h)}\right)$$

- Type III

$$N_e = N_0 - W\left(\frac{(a\ N_0)h\ N_0 \exp(-(a\ N_0)\ (P\ T - h\ N_0))}{((a\ N_0)h)}\right)$$

Where $\boldsymbol{N_e}$ is the number of prey consumed, $\boldsymbol{N_0}$ is the initial prey density, $\boldsymbol{a}$ is the instantaneous attack rate of the predator, $\boldsymbol{h}$ is the handling time of the predator, $\boldsymbol{P}$ is the density of the predator (here always 1), $\boldsymbol{T}$ is the experimental period (here always 24 hours), and $\boldsymbol{W}$ is the Lampert W function (see Bolker 2008 for further details). Both equations were applied at each of the three temperature treatments.

I calculated the functional response curve from each model fit for each individual pairwise combination. I then used the package "bbmle" (Bolker 2008) to apply Akaike Information Criteron (Akaike 1974) to test both equations for goodness-of-fit and identify the most parsimonious model (after Rall *et al.* 2011; Lang *et al.* 2012), which could then be used to test my first hypothesis.

Once that model was identified, I then fitted a model which incorporates the effect of temperature into the functional response, and allows for comparison of 'activation energies' in attack rate and handling time by adding $\boldsymbol{E_a}$ as the intercept of the attack rate activation energy and $\boldsymbol{E_h}$ as the intercept of the handling time activation energy (i.e. by analogy with enzyme kinetics, the responses of attack rate and handling time to temperature). This was done in order to remove the temperature effect per se, to allow comparison of predation among different temperatures.

Mean individual body mass data for each invertebrate taxon were available from Surber samples of the benthos of Broadstone Stream in the 1970s, 1980s, 1990s & 2000s (A. Hildrew, C. Townsend, K. Schofield, J.Lancaster, G.Woodward & K.Layer). I then calculated biomass (mg) (B) by multiplying prey abundance (N) by mean body mass (mg) (M), and compared the saturation points for each pairwise predator-prey functional response using the "confint" function in the "bblme" package (Bolker 2008), which tests if 95% confidence interval overlaps were significant. I could then observe if functional response curve asymptotes significantly differed between acid-tolerant and acid-sensitive prey biomass for each predator.

To enable comparison of predation (i.e. percentage of initial prey consumed) across density treatments between acid-tolerant and acid-sensitive prey, I used Zar's arcsin transformation (Zar 1996). This transformation was used as it is more effective than standard arcsin-transformation at

stabilising the extremes of distribution .***X*** denotes number of prey surviving after 24 hours of predator exposure, and ***n*** denotes initial prey density:

$$p' = \arcsin\sqrt{\frac{X+\frac{3}{8}}{n+\frac{3}{4}}} \text{ (Zar, 1996)}$$

I then performed a repeated-measures ANOVA with categorical prey tolerance using the statistical programing language R (R Development Team 2012) loaded with the package "nlme", to test for differences in prey mortality in line with my second hypothesis. Prey ID, initial prey density and temperature were fitted as fixed factors, and categorical prey tolerance (i.e. acid-tolerant or acid-sensitive) was fitted as a random effect. Since the models were unbalanced because of differences in prey density, I used a restricted maximum likelihood method to account for this, after Crawley (2012).

Results

Functional responses

Comparison of varying functional response models revealed that the Type III functional response (see Equation 2 in Methods, Statistical Analysis) proved to be the best fit across all the predator-prey interaction experiments (see Appendix 2 for AIC comparisons), in line with my first hypothesis. Consequently, all further analyses on functional response parameters (i.e. handling times, encounter rate and attack rate) were performed using that model. This Type III functional response was exhibited for all predators across the both resident invertebrate guild and the acid-sensitive simulated invasive (Figs 2.4, 2.6, 2.8).

When these responses were expressed as the biomass of prey consumed (see Figs 2.5, 2.7, 2.9), using the asymptotes of each individual pairwise predator-prey combination, the Type III functional response curves did not have significantly different 95% confidence intervals (see Appendix 3) for all three predators. Effectively, this indicates that there was no difference in gut capacity between acid-sensitive and acid-tolerant prey species.

Prey mortality

For each predator, I used LMEMs to examine prey mortality. This revealed increased vulnerability to *C.boltonii* of the acid-sensitive mayflies when compared to the acid-tolerant stoneflies (LMEM; see Table 2.1). There was also a significant two-way interaction between prey ID and prey density.

The LMEM comparisons of prey vulnerability to both *S.fuliginosa* and *P.conspersa* showed no significant results in terms of prey ID, indicating the apex invertebrate *C.boltonii* was the only predator to distinguish between species of different acid tolerance. This can also be seen in the parameters simulated by the Type III functional response model (see Appendix 4), with significantly higher attack rates apparent on acid-sensitive mayflies than acid-tolerant stoneflies for *C.boltonii*.

Temperature response

Across all three temperature treatments for all predator-prey pairwise combinations, there was no difference in the shape of the functional response (i.e. they were consistently of Type III). In terms of the individual predators, *Cordulegaster boltonii* showed a significant increase in attack rate and a significant decrease in handling time for all four prey species as temperature increased (see Table 2.2). There was also a significant effect of temperature on prey mortality, and a significant interaction between temperature and prey density (LMEM; see Table 2.1). However, neither *P.conspersa* nor *S.fulignosa* showed any consistent pattern in either attack rate or handling time as temperature increased (see Table 2.2).

Tables & Figures

Table 2.1. Repeated Measures ANOVA with restricted maximum likelihood method (REML) and nested output of changes in prey mortality with *C.boltonii* as the main predator. Model values shown are only for fixed factors. Variances due to the categorical random effect (prey acid tolerance) have been partitioned in the model and are therefore not shown).

	P	**t-value**
Prey ID	> 0.05	-0.379628
Prey Density	< 0.01	-2.865195
Temperature	< 0.05	1.830614
Prey ID x Prey Density	< 0.001	12.546566
Prey ID x Temperature	> 0.05	1.282232
Prey Density x Temperature	< 0.001	5.667917
Prey ID x Prey Density x Temperature	> 0.05	1.273936

Table 2.2. Parameters and standard errors (SE) estimated by the temperature dependent model, accounting for the effect of temperature. a_0 is the intercept of the attack rate, h_0 is the intercept of the handling time, E_a is the intercept of the attack rate activation energy and E_h is the intercept of the handling time activation energy. Note that model could not be fitted to *S.fulignosa-L.nigra* (Level of significance: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$).

Predator	**Prey**	**a_0**	**SE**	**h_0**	**SE**	**E_a**	**SE**	**E_h**	**SE**
C.boltonii	*B.rhodani*	0.17***	0.02	0.12***	0.01	0.75**	0.26	-0.23	0.12
C.boltonii	*L.nigra*	0.04***	0.01	0.24**	0.09	1.94***	0.44	0.26	0.41
C.boltonii	*N.pictetii*	0.08***	0.02	0.22***	0.04	1.60***	0.39	-0.07	0.34
C.boltonii	*P.submarginata*	0.21***	0.03	0.10***	0.01	0.83***	0.22	-0.20	0.11
S.fuliginosa	*B.rhodani*	0.02	0.02	1.96	1.03	0.68	2.19	-1.22	1.07
S.fuliginosa	*L.nigra*	-	-	-	-	-	-	-	-
S.fuliginosa	*N.pictetii*	0.005*	0.002	0.34	0.64	-1.01	0.72	-1.91	2.49
S.fuliginosa	*P.submarginata*	0.01*	0.006	0.83	0.43	1.33	0.79	-0.32	0.82
P.conspersa	*B.rhodani*	0.07*	0.02	0.98***	0.20	0.18	0.73	-0.25	0.34
P.conspersa	*L.nigra*	0.06*	0.02	0.81***	0.17	-0.24	0.71	-0.57	0.36
P.conspersa	*N.pictetii*	0.04*	0.01	0.50**	0.16	1.79**	0.64	-0.44	0.53
P.conspersa	*P.submarginata*	0.03*	0.01	0.74***	0.20	0.78	0.75	-0.29	0.48

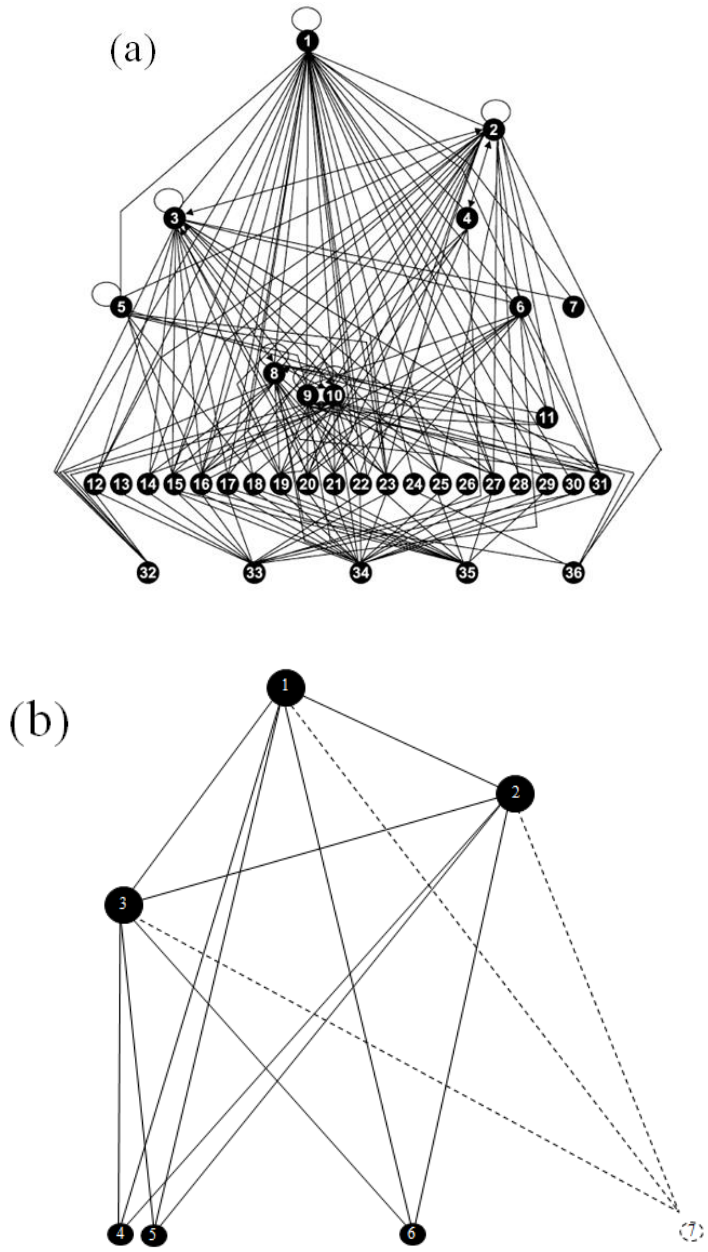

Figure 2.1. (a) Summary binary food web for Broadstone Stream (redrawn from Woodward *et al.* 2005a) and (b) Experimental species highlighted on conceptual food web subset for Broadstone Stream. Solid black nodes represent recorded species in the system, with circle area proportional to secondary production, solid lines represent observed feeding links, dotted circles represent potential acid-sensitive invaders and dotted lines represent potential feeding links. Key to species: 1. *Cordulegaster boltonii*; 2. *Sialis fuliginosa*; 3. *Plectrocnemia conspersa*; 4. *Nemurella pictetii*; 5. *Leuctra nigra*; 6. *Paraleptophlebia submarginata*; 7. *Baetis rhodani*.

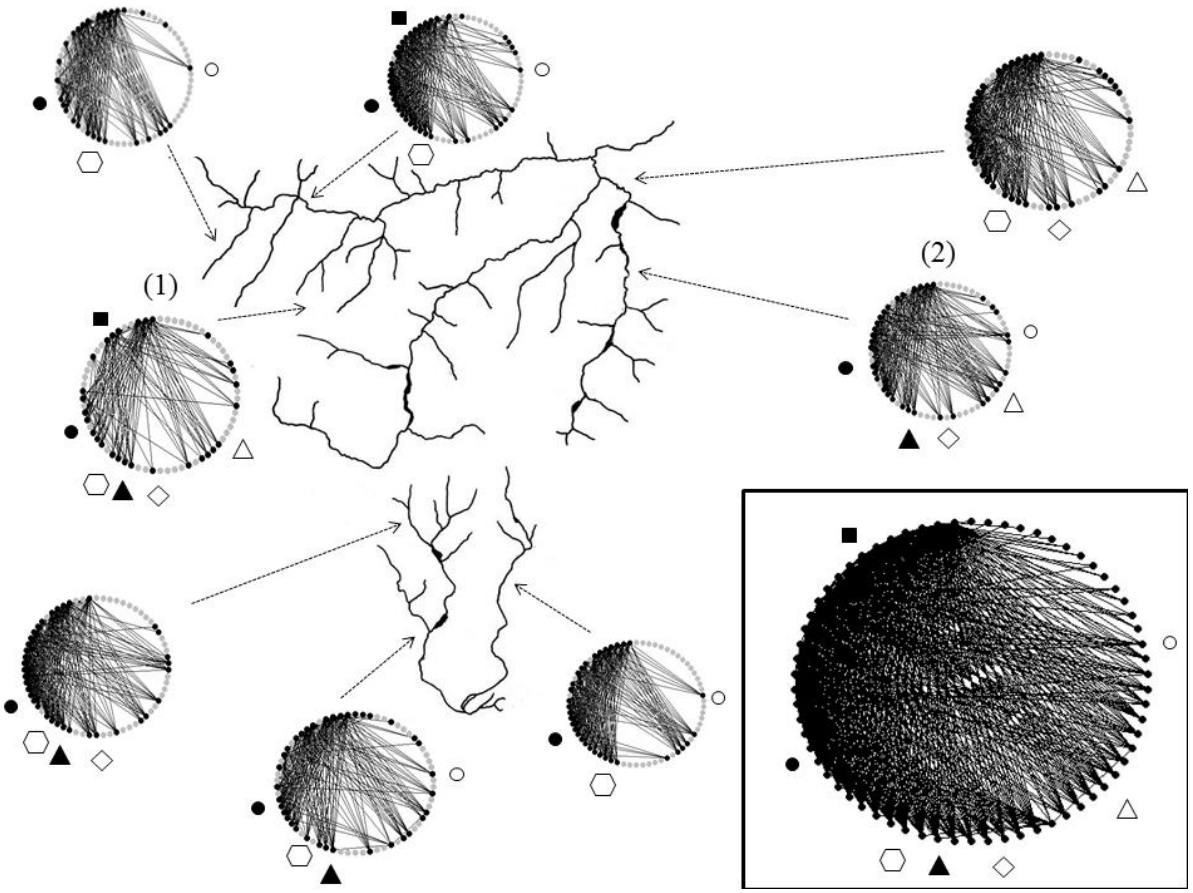

Figure 2.2. Individual networks of some interconnected stream food webs from the Ashdown Forest catchment (Broadstone Steam marked as 1, and Withyham Stream as 2), with the regional food web in bottom-right box. Each individual web contains the same number and positioning of nodes as in the regional food web; small black nodes represent taxa present in depicted web, and small grey nodes represent taxa absent from stream web but present in regional web. Black lines indicate feeding links. When experimental predators/prey are present in any given web, they are denoted by either solid black symbols (predators) or open symbols (prey) on the circumference of the network. See Townsend *et al.* (1983) for names and physicochemical information of specific streams.

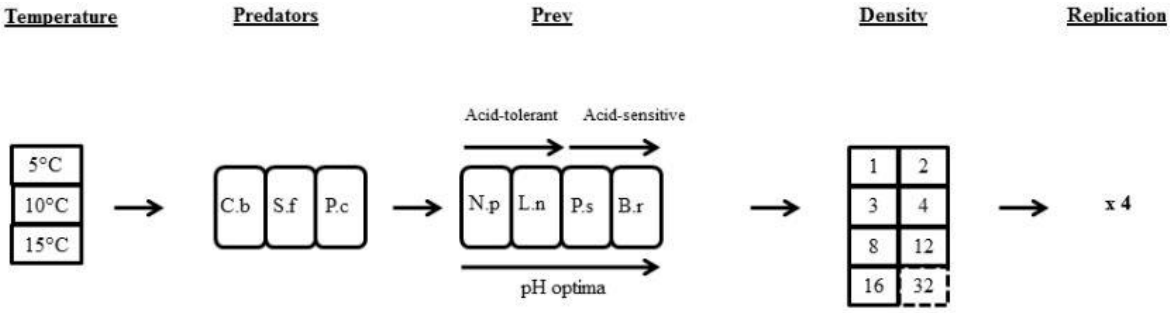

Figure 2.3. Experimental design for predator-prey interaction experiment. C.b = *Cordulegaster boltonii*, S.f = *Sialis fuliginosa*, P.c = *Plectrocnemia conspera*, N.p = *Nemurella pictetii*, L.n = *Leuctra nigra*, P.s = *Paraleptophlebia submarginata*, B.r = *Baetis rhodani*. Dotted line on 32 individuals under 'Density' column indicates this treatment only used for *C.boltonii-P.submarginata* & *C.boltonii-B.rhodani* experiments. Total *n* = 1008.

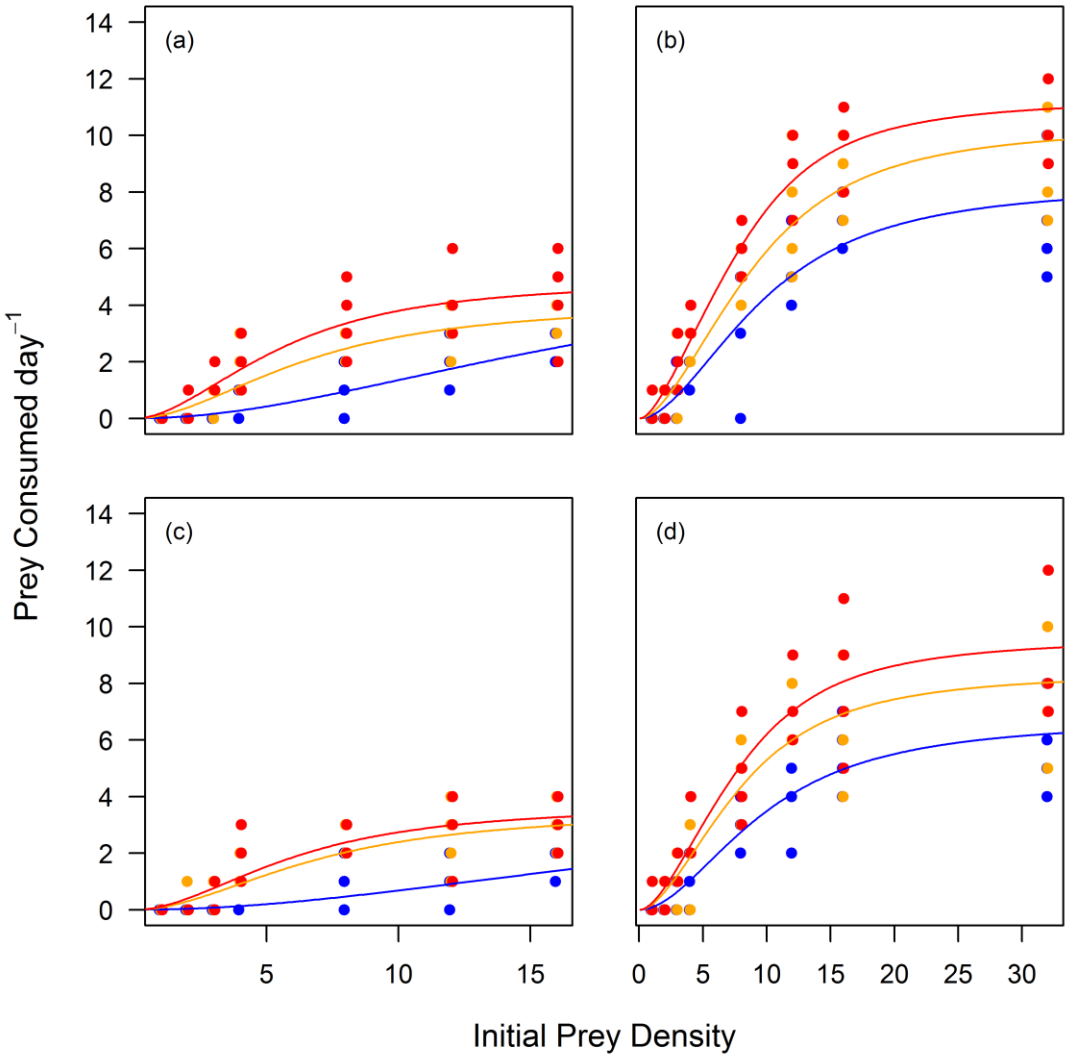

Figure 2.4. Functional response curves for *C.boltonii* for individual prey eaten per predator, with initial density of individual prey on the x-axes and individual prey eaten on the y-axes. Each pane corresponds to a different prey species: (a) *N.pictetii* (b) *P.submarginata* (c) *L.nigra* (d) *B.rhodani* Three temperature treatments are indicated by blue (5°C), orange (10°C) and red (15°C) curves. Note the extended x-axes on panels (b) and (d) due to additional initial prey density treatments.

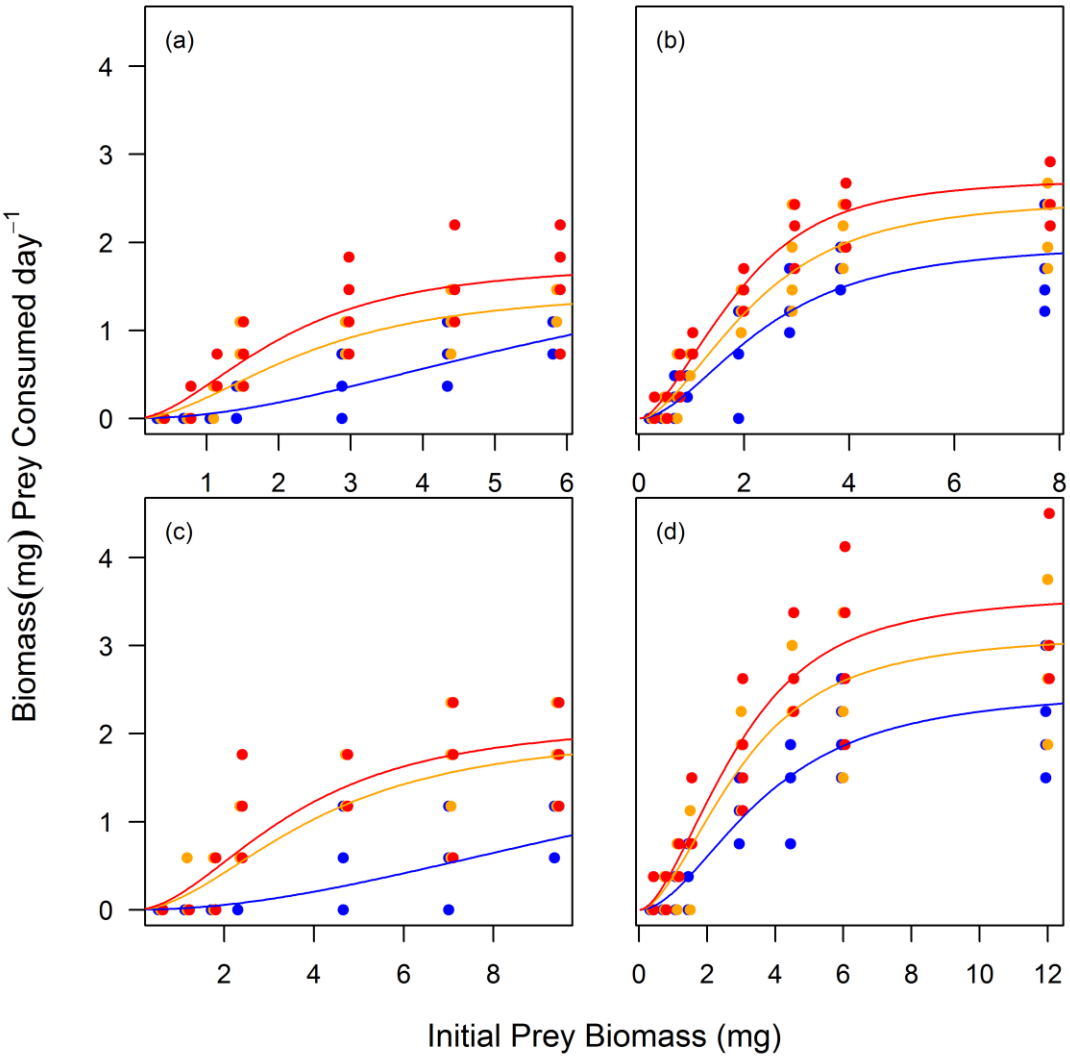

Figure 2.5. Functional response curves for *C.boltonii* for prey biomass in milligrams eaten per predator, with initial biomass of prey on the x-axes and biomass eaten on the y-axes. Each pane corresponds to a different prey species: (a) *N.pictetii* (b) *P.submarginata* (c) *L.nigra* (d) *B.rhodani*. Three discrete temperature treatments are indicated by blue (5°C), orange (10°C) and red (15°C) curves. Note the extended x-axes on panels (b) and (d) due to additional initial prey density treatments.

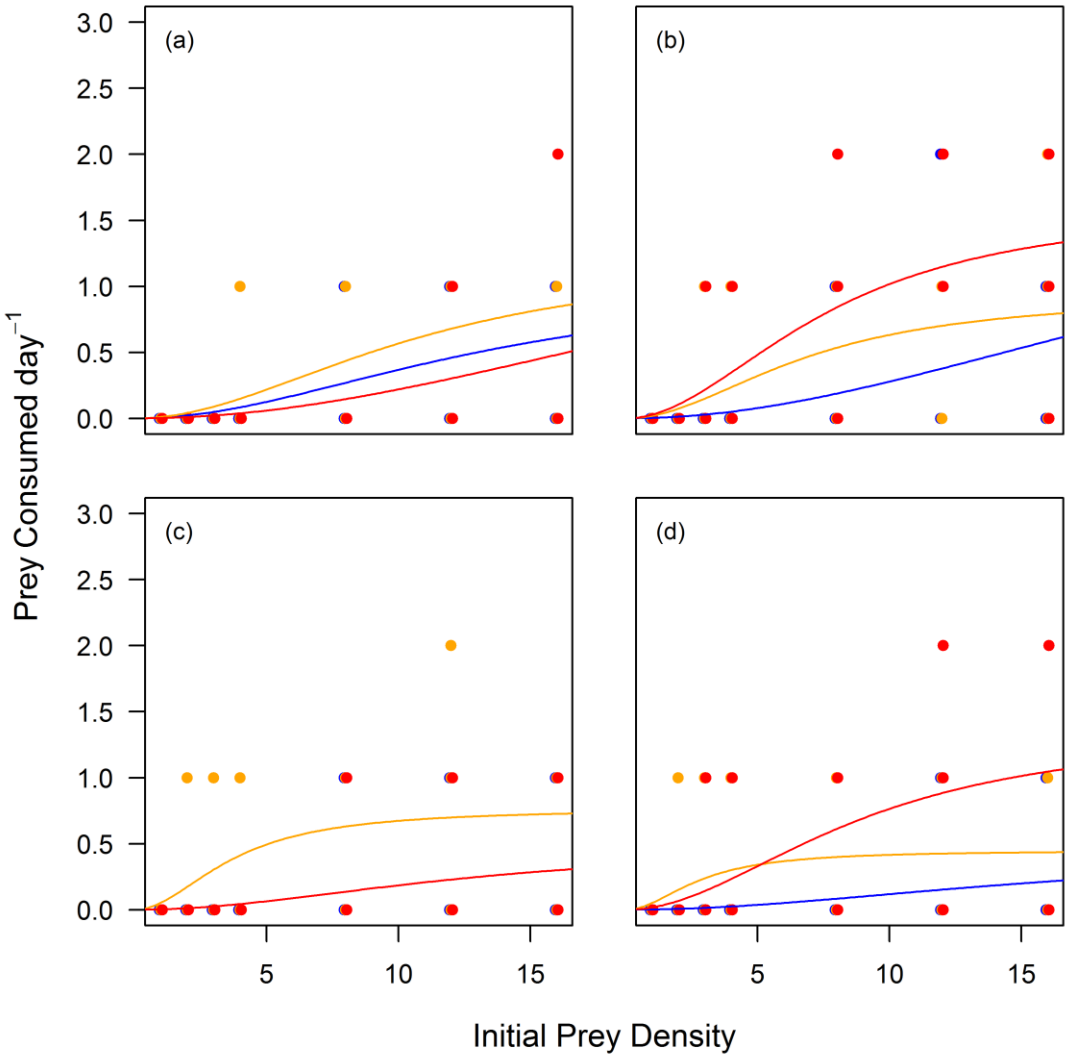

Figure 2.6. Functional response curves for *S.fuliginosa* for individual prey eaten per predator, with initial density of individual prey on the x-axes and individual prey eaten on the y-axes. Each pane corresponds to a different prey species: (a) *N.pictetii* (b) *P.submarginata* (c) *L.nigra* (d) *B.rhodani*. Three discrete temperature treatments are indicated by blue (5°C), orange (10°C) and red (15°C) curves.

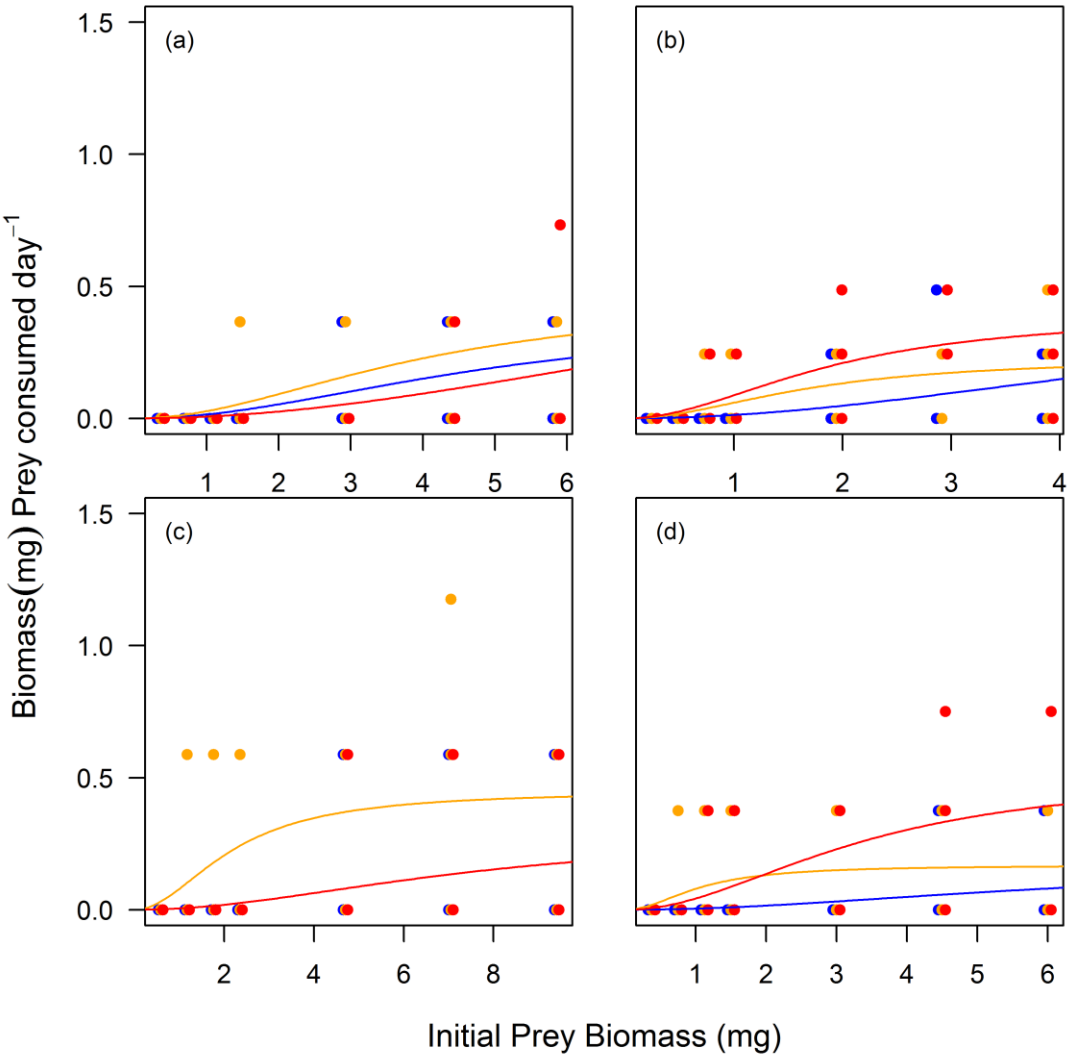

Figure 2.7. Functional response curves for *S.fuliginosa* for prey biomass in milligrams eaten per predator, with initial biomass of prey on the x-axes and biomass eaten on the y-axes. Each pane corresponds to a different prey species: (a) *N.pictetii* (b) *P.submarginata* (c) *L.nigra* (d) *B.rhodani*. Three discrete temperature treatments are indicated by blue (5°C), orange (10°C) and red (15°C) curves.

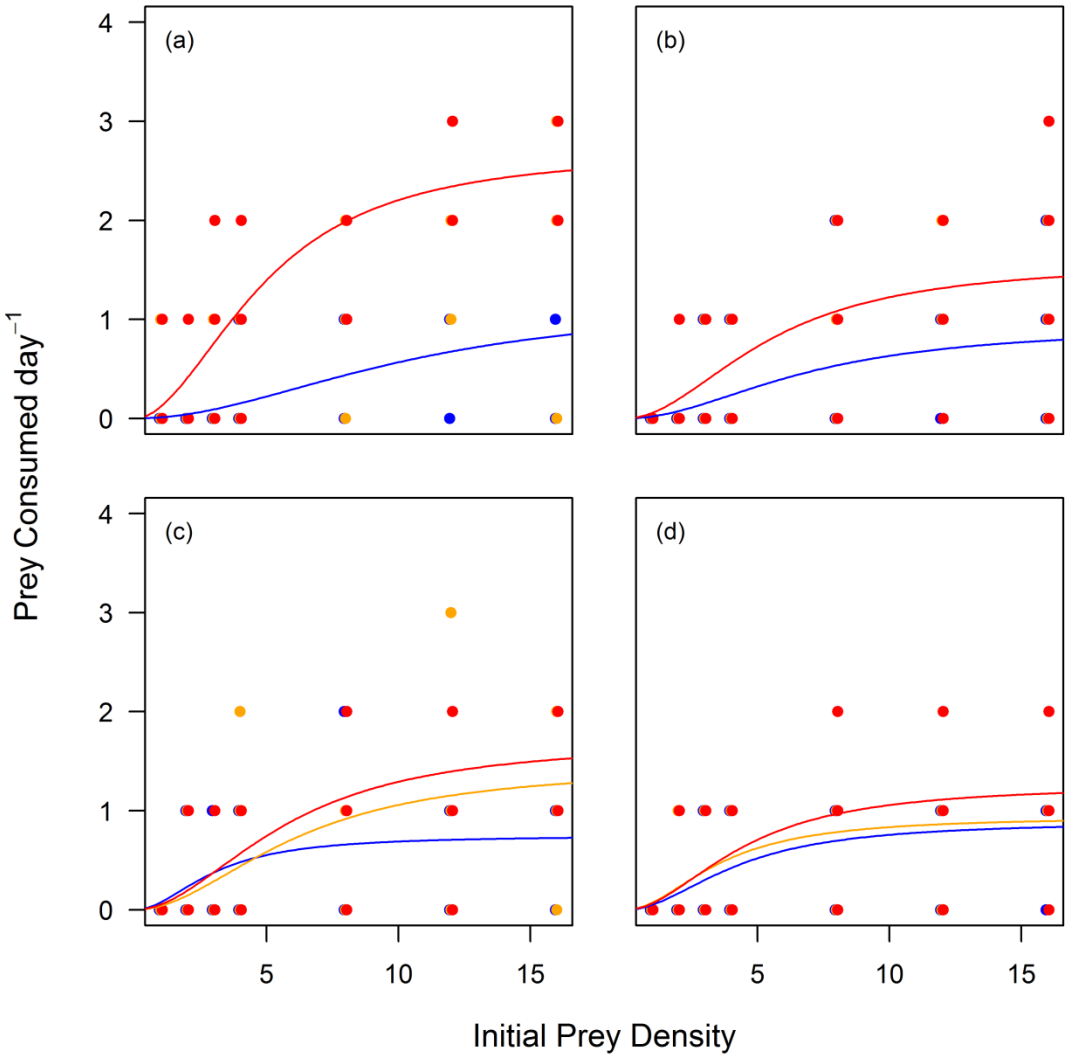

Figure 2.8. Functional response curves for *P.conspersa* for individual prey eaten per predator, with initial density of individual prey on the x-axes and individual prey eaten on the y-axes. Each pane corresponds to a different prey species: (a) *N.pictetii* (b) *P.submarginata* (c) *L.nigra* (d) *B.rhodani*. Three discrete temperature treatments are indicated by blue (5°C), orange (10°C) and red (15°C) curves.

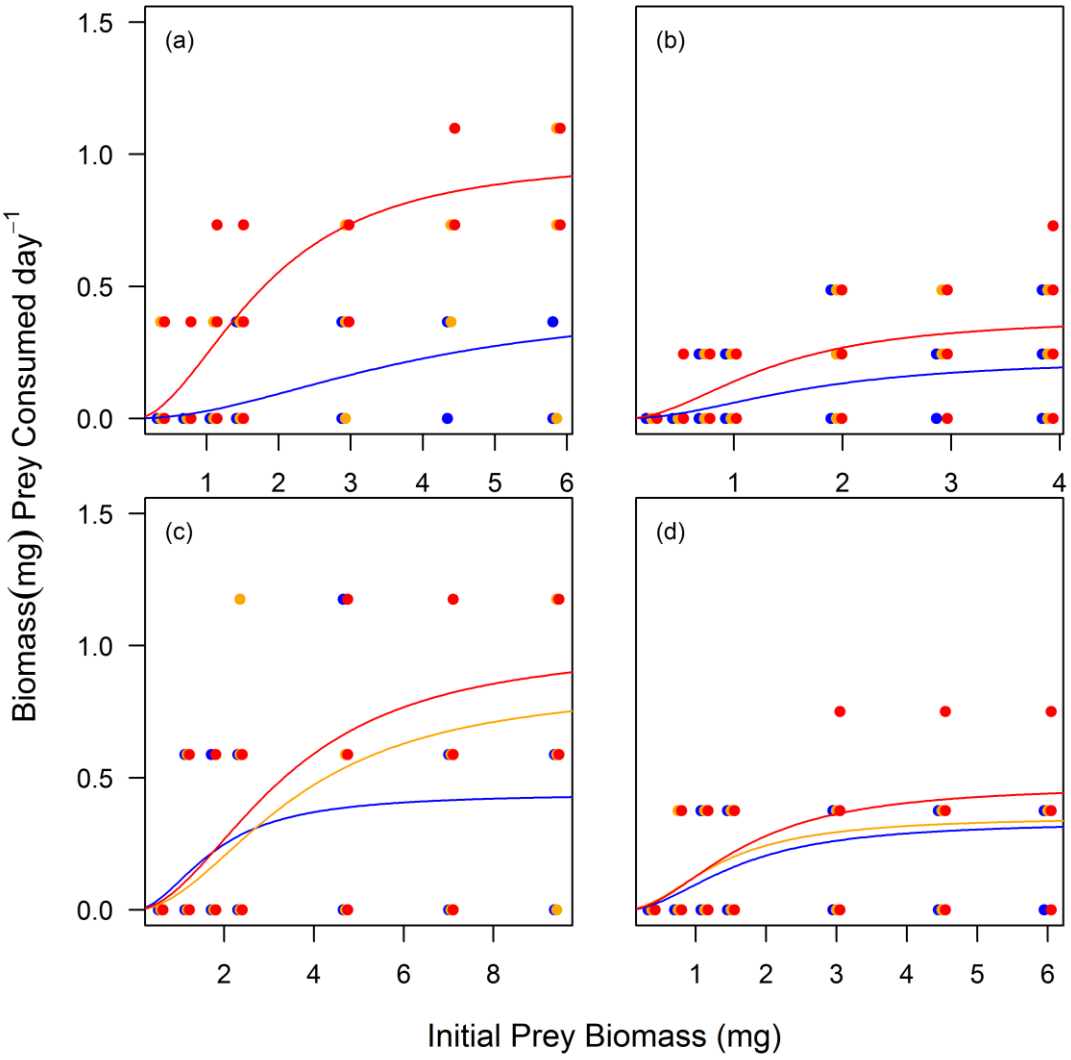

Figure 2.9. Functional response curves for *P.conspersa* for prey biomass in milligrams eaten per predator, with initial biomass of prey on the x-axes and biomass eaten on the y-axes. Each pane corresponds to a different prey species: (a) *N.pictetii* (b) *P.submarginata* (c) *L.nigra* (d) *B.rhodani*. Three discrete temperature treatments are indicated by blue (5°C), orange (10°C) and red (15°C) curves.

Discussion

A potential population-level stabilising effect, the Type III functional response, was found for all predator-prey combinations and across all temperatures, consistent with my first hypothesis, although the attack rates and handling times varied among these different combinations. This implies that, at low densities, the predators are exerting a density-dependent stabilising effect on prey, which is consistent with other studies on the functional response of predators (e.g. McCann *et al.* 2005; Brose *et al.* 2006b). Density-dependent predator-prey interactions such as these could allow for low levels of a prey population to persist, as predation is constrained by encounter rate in these ranges (Spiers *et al.* 2000), due to factors such as refugia availability (Hildrew *et al.* 2004). However, if populations increase, refugia availability correspondingly decreases, the encounter rates between predators and prey in turn increase and this could, ultimately, lead to predator-mediated 'damping' of any large-scale rise in population levels (Woodward & Hildrew 2001; Rall *et al.* 2008). This could act in combination with strong apparent competition pressures, which result from a prevalent generalism among the entire predator guild, across all trophic levels, such as seen in Broadstone (Woodward *et al.* 2005a). I investigate this avenue further in Chapter Three.

Paraleptophlebia submarginata and the other acid-sensitive mayfly in this study, *B.rhodani*, were significantly more vulnerable than acid-tolerant stoneflies to predation by *C.boltonii,* which is consistent with my second hypothesis. *Cordulegaster boltonii* is, on average, an order of magnitude larger than any other invertebrate predator in the system (Woodward & Hildrew 2001), with one of the fastest attack responses in the animal kingdom (Pritchard 1965). It has a gut capacity far in excess of *P.conspersa* or *S.fuliginosa (*Woodward *et al.* 2005a), and has the ability to handle both large and small prey items via the flexibility of its labial mouthparts (Corbett 1999). In addition, *C.boltonii* is a cryptic ambush predator with a "sit-and-wait" strategy for prey

capture (Woodward & Hildrew 2001). As a result of a combination of a stabilising response at low densities, and this significantly increased vulnerability to the largest invertebrate predators in the system, compared to resident species, acid-sensitive species attempting to colonise the system could face strong resistance from the resident predator guild.

The universality of the Type III response does not alter across differing temperature regimes, indicative of seasonal shifts, suggesting that the predator response is always stabilising irrespective of temperature. However, the magnitude of this density-dependent stabilisation may change with temperature (e.g. maximum ingestion rates increase with temperature as a function of metabolic rate (Brown *et al.* 2004)), consistent with my third hypothesis. Given that the population density of both prey and predator species will cycle on an annual basis, due to recruitment, growth and emergence from the system (all experimental species have winged imagines), this suggests there will be seasonal changes in the magnitude of top-down predation pressure. In addition, the saturation points of each pairwise predator-prey response did not differ significantly between acid-tolerant and acid-sensitive prey species, indicating consistent asymptotes across temperature treatments. However, I did observe alterations in response parameters as temperature increased; an increase in attack rate, and a decrease in handling time, for the largest invertebrate predator *C.boltonii,* which could lead to an increase in the accelerating per capita consumption function of a Type III response. These findings are broadly consistent with recent functional response studies (Petchey *et al.* 2010; Vucic-Pestic *et al.* 2011; Lang *et al.* 2012).

Correspondingly, this effect of temperature increase could reduce survival of prey populations in high summer stream temperatures, when recruitment is at its peak. Predation has been shown to be most intense in Broadstone during the summer months (Woodward *et al.* 2005a), which could mean that populations attempting to establish will be faced with strong top-down predator pressure as a result of increased attack rates, combined with the ‘damping’ population effect of a

stabilising functional response. While Type III responses can be destabilising at high densities, the potential and realised predation rates in Broadstone can be markedly different. Woodward & Hildrew (2002a) used microcosm trials to demonstrate that *C.boltonii* rarely, if ever, achieves gut satiation in the field, by comparing natural gut contents with prey intake rates from laboratory trials. This suggests that the accelerating phase of the response will not reach the point where it can be destabilising, due to low natural densities. Instead, it is likely that the rapidly accelerating per capita consumption phase of the response (in effect a form of positive feedback) will result in high mortality of prey populations before that point is reached, hence why Type III responses are widely seen as stabilising (Murdoch & Stewart-Oaten 1975; Aljetlawai *et al.* 2004; Rall *et al.* 2008; Kalinkat *et al.* 2013).

This population-level stability is not necessarily mutually exclusive from stabilising factors at higher levels of organisation. It is well-established that stable food webs are less influenced by perturbations, such as environmental stressors (e.g. temperature, drought, eutrophication) and invasive species (e.g. MacArthur 1955; McCann 2000; Rall *et al.* 2008; Layer *et al.* 2010b; Ledger *et al.* 2012). An inherent resistance to invaders and re-colonisers, governed by predator-prey interactions, could explain why populations of acid-sensitive species fail to establish sizeable populations in streams that are otherwise chemically suitable, and contribute to overall network stability. As the vast majority of engulfing predators in aquatic systems are gape-limited (Aljetlawi *et al.* 2004; Brose *et al.* 2006a), an invasion which also involves an increase in body size may be the only way it could be successful, thanks in part to 'size refugia' - a common characteristic in many ecosystems (Bechara *et al.* 1993; Chase 1999; Woodward *et al.* 2008; Yvon-Durocher *et al.* 2011). It has been widely demonstrated that body size plays a key role in the structure and function of ecological networks (Cohen *et al.* 2003; Jennings & Mackinson 2003; Woodward *et al.* 2005b; Brose *et al.* 2006a; Jacob *et al.* 2011; O'Gorman *et al.* 2012). In Broadstone, the only successful invaders in the system since extensive studies began in the 1970s

have been the apex predators *C.boltonii* and *Salmo trutta* L. (Woodward & Hildrew 2001; Woodward *et al.* 2005a; Layer *et al.* 2011), which both represented at least an order of magnitude increase in average body size on the previous apex predator. This increase in large invertebrate predators could potentially act as an energy conduit within the system, linking increasing basal resource availability/productivity (which I show in Chapter Five to be increasing, through rising rates of microbial decomposition, as acidity ameliorates) and larger predator species (I show in Chapter Four that energy transfer further up the network has cumulated in a new apex vertebrate predator) helping to sustain new trophic levels of larger species as chemical conditions improve.

However, there is one caveat. The increased mortality of acid-sensitive mayflies with *C.boltonii* was not shown in the results from the other predatory species - *S.fuliginosa* and *P.conspersa*. Despite this, these two species do still exhibit significant density-dependent Type III responses with all four prey species, and they account for a large proportion of the predatory interactions within the system (Woodward *et al.* 2005a). If the majority of antagonistic trophic links within any given network exhibit this stabilising response, as is potentially the case in Broadstone, it is likely the system will be stable at a higher level of organisation than at the population level alone.

Essentially, while the presence of acid-sensitive species abundant in circumneutral streams might be possible in recovering streams at low densities, the response of resident predators shown here could act to constrain any increase in prey density and confer stability at the population level, which is critically dependent on predator functional responses (Williams & Martinez 2004; Brose *et al.* 2006b; Rall *et al.* 2008). This effect is present across a variety of predators and prey, and, in combination with stabilising effects at higher levels of organisation (e.g. Layer *et al.* 2010b; 2013), offers a potential explanation as to why acidified aquatic communities might be resistant to re-colonisation by species previously excluded by physicochemical conditions, despite large-scale and long-term environmental recovery.

Chapter Three: Simulating indirect interactions between acid-tolerant and acid-sensitive species

Abstract

The widespread chemical recovery of fresh waters from the anthropogenic acidification of the 19th and 20th centuries has not yet been reflected in similarly substantial response in the biota. One possible explanation for this is that aquatic communities are intrinsically resistant to invasion, or re-colonisation, by acid-sensitive species, even as the physicochemical conditions ameliorate. This resistance could be attributable to processes at several levels of organisation, including interactions between and among species, which are increasingly recognised as having a crucial role in the structuring of ecological communities. Here I investigate two separate indirect predator-prey interactions (building on the pairwise interactions dealt with in the last Chapter); those of apparent competition and behavioural effects (i.e. involving three species – and cues of a fourth species). I used experimental manipulations of two invertebrate prey species, one acid-tolerant and one acid-sensitive, in the presence of a large invertebrate predator, hypothesising that apparent competition between the prey would lead to increased mortality of the more acid-sensitive of the two. In addition, chemical cues from a vertebrate predator (brown trout) were used to observe whether any behavioural effects arising from avoidance would alter this apparent competition. I found that at, high densities of both prey, the acid-sensitive species suffered from increased relative mortality, but that this mortality was not altered by any form of behavioural response to fish clues. Thus, apparent competition among prey could contribute to inertia in the recovery of an acid-sensitive assemblage, as the latter is inhibited or excluded by species

interactions. This could explain the observed 'stability' of acid stream communities, and constitute an intrinsic 'structural resistance' of these networks of interactions to biological recovery.

Introduction

The factors that regulate and stabilise the complex natural communities have long been a key focus for ecologists searching for so-called "devious strategies" that enable such systems to persist, contrary to theoretical predictions that simplicity confers stability (May 1972; 1973). Among such factors are species interactions, the stability of which have long been the focus of ecology (Begon *et al.* 2009). For instance, direct competitive interactions, such as competition for resources, have long been thought to be very important in the structuring of communities (McAuliffe 1984; Allan 1995; Bonsall & Hassell 1997; Kohler & Wiley 1997) and are a major area of interest in this regard. However, the dynamics of indirect competition are less well understood (Holt 1977; Holt & Lawton 1994). Indirect interactions arise when the link between two species is mediated by another species, such as when two prey species share a common predator, a common phenomenon in natural communities (Schmitt 1987; Holt & Lawton 1994; Müller & Godfray 1997; Chaneton & Bonsall 2000). This indirect link between prey can create indirect interspecific competition (Williamson 1957), or, more accurately, apparent competition (Holt 1977). The two prey species compete for 'enemy-free space', which will result in either the exclusion of one prey species or a competitive co-existence – similar to resource-mediated direct competition (Jeffries & Lawton 1984; Chaneton & Bonsall 2000). This can potentially limit the absolute number of co-existing prey species in a food web (Holt & Lawton 1993).

Apparent competition has the potential to stabilise a community, by increasing inherent resistance to structural changes or species invasions. Indirect effects such as this have been shown to dampen the dynamics of otherwise destabilising strong consumer-resource interactions (McCann *et al.* 1998). Quantitative food webs are useful model systems for assessing the impacts of such indirect interactions (Schmitt 1987) and understanding the role of apparent competition is vital for our understanding ecological network dynamics (van Veen *et al.* 2006). It has been suggested (Lawton 1986; Chaneton & Bonsall 2000) that apparent competition may exclude invaders/recolonisers from establishing populations in resident prey assemblages, especially when the shared predator is an extreme dietary generalist (Woodward & Hildrew 2001). This is a common trait in freshwater food webs, where diets are largely governed by gape limitation (Brose *et al.* 2006a) and encounter rate (Woodward *et al.* 2005a; Woodward *et al.* 2010b), and apparent competition is seemingly especially prevalent in aquatic systems (Mithen & Lawton 1986; Huang & Sih 1990; Menge 1995). This is relevant to community stability when considering one of the major recent environmental perturbations in freshwater systems over the last century: acidification.

Since the cause of acidification of European and North American fresh waters in the 20th century was diagnosed (e.g. Flower & Battarbee 1983), legislation to reduce acidifying emissions was introduced and implemented and chemical recovery of acidified sites has been widespread (see Chapter One; UKAWMN 2010). However, this has not translated to a general corresponding biological recovery i.e. the aquatic taxa lost from local ecosystems have not recolonised at the same rate (there is however some evidence for recovery - see Tipping *et al.* 2002; Layer *et al.* 2011). There have been a number of hypotheses to explain this, such as recurrent acidic episodes, limitations on species dispersal and intrinsic food web resistance (see Chapter One for details), but there is no universal consensus as to why this might be, and more than one mechanism may be operating. In the context of the last hypothesis, Layer *et al* (2010b; 2013) demonstrated that

acidified food webs are more robust, and therefore more structurally resistant, than those of circumneutral systems. This stability may be present at different levels of organisation (i.e. individual, population, communities), which are not necessarily mutually exclusive from each other. In this chapter, I investigate experimentally whether indirect interactions between predators and prey, at the basic network level, are at least partially responsible for the observed lags or hystereses in biological recovery of the food web, using experiments on the biota of an acidified model system, Broadstone Stream, in south-east England. Broadstone is a recovering acid stream and has been extensively studied since the 1970s (see Chapter One). Its community has one of the most well-documented food webs published to date (Hildrew *et al.* 1985; Woodward & Hildrew 2001; Schmid-Araya *et al.* 2002; Woodward *et al.* 2005a; Hildrew 2009; Layer *et al.* 2011) and is an ideal system for studying the ecological consequences of deacidification. Contrary to the general trend of biological lag in response to amelioration of acidification, Broadstone has seen several irruptions or invasions of successively larger predators since the 1970s, all exhibiting extreme dietary generalism, cumulating in the invasion of brown trout (*Salmo trutta* L.) around the turn of the century (Woodward *et al.* 2005a; Layer *et al.* 2011). The presence of these generalist predators has led to an increase in the potential for apparent competition in Broadstone (Woodward & Hildrew 2001), and may explain why, despite the amelioration of acidity, there has been no overall re-establishment of an acid-sensitive fauna lower in the food web. It has been invoked as an explanation for the sporadic appearance of a leptophlebiid mayfly species (*Paraleptophlebia submarginata* Stephens) in low densities in the network that has, as yet, never established a persistent and sizeable population, despite optimal chemical conditions and abundant basal resources. However, apparent competition in the system has yet to be tested experimentally, and experimental studies of apparent competition are rare in general (Chaneton & Bonsall 2000).

I have previously shown (Chapter Two) that acid-sensitive species are more vulnerable to predation than acid-tolerant species, with attack rates by large invertebrate predators markedly

higher on potential invaders than on acid-tolerant residents. Holt & Lawton (1994) suggested that high attack rates may lead to exclusion from a system via indirect interactions, if the invading prey species closely resemble residents in niche dimensions that determine vulnerability to predation. In Broadstone, it is likely that this niche dimension is body size and microhabitat use, which combine to determine refugia availability and predation rates (Woodward & Warren 2007), as this has a significant influence on the mortality of acid-tolerant residents (Hildrew & Townsend 1977). As apparent competition is a well-known density-mediated indirect effect (Montoya *et al.* 2009), and density is important in predator-prey dynamics in Broadstone (Chapter Two; Hildrew & Townsend 1977; Hildrew *et al.* 2004), it could well be that this indirect effect is occurring within the Broadstone community. However, it is also possible that other, more subtle, indirect interactions are important; specifically, behavioural factors could be influential in predator-prey dynamics (Holt & Lawton 1994; van Veen *et al.* 2006; Montoya *et al.* 2009).

Behavioural ecology is increasingly recognised as playing a significant role in our understanding of food web structure and dynamics (e.g. Beckerman *et al.* 1997; Luttberg & Kirby 2005; Petchey *et al.* 2008; Ings *et al.* 2009). Behavioural-level effects arise at a different, individual-based, level of organisation to those of population-level direct effects (Schmitz *et al.* 1997), but both may play a role in food web dynamics, and this modification of predator-prey interactions by behavioural cues of risk perceived by individuals could have consequences at the population and network level and thus for dynamic stability (McCann 2000; Neutel *et al.* 2002; Berlow *et al.* 2004; Montoya *et al.* 2009).

In most natural systems, prey are vulnerable to multiple predators (Pimm *et al.* 1991), and this is the case in Broadstone (Woodward *et al.* 2005a; Hildrew 2009). Thus, behavioural avoidance of any one predator could lead to increased vulnerability to another (McIntosh & Peckarsky 1999). For example, baetid mayflies flee into the water column in the presence of large invertebrate predators, but retreat into the benthos when in the presence of fish (Wooster & Sih 1995) - if both

species are present, then a form of behavioural 'compromise' is needed to ensure survival, as there it is unlikely that avoidance traits are 'cumulative' in nature (McIntosh & Peckarsky 1999). Moreover, as acidity ameliorates, acid-sensitive mayflies and other species with similar tolerances have begun to appear in the system. For example, there is evidence of sporadic leptophlebiid colonisation of the stream (Woodward & Hildrew 2001; Layer *et al.* 2011) while baetid mayflies have also been found very sporadically, particularly in summer (A. Hildrew, personal communication).The resident, acid-tolerant prey species are predominantly stoneflies – which are far less vulnerable to trout predation than mayflies (Allen 1982; McPeek & Peckarsky 1998). I have also shown that two of the most abundant of these acid-tolerant species do not exhibit behavioural reaction to the presence of predators (Chapter Four; Layer *et al.* 2011), but several mayflies and other acid-sensitive species, such as gammarids, often reduce their movement and feeding activity in response to behavioural cues triggered by the presence of kairomones (Cowan & Peckarsky 1994; McIntosh & Peckarsky 1996; Abjornsson *et al.* 2001). As a result of these traits, mayflies can be present in higher densities in streams with fish than those without, thanks in part to enhanced survival as a result of behavioural mediation of activity, despite their apparent increased vulnerability to fish predation (Peckarsky & McIntosh 1999; Peckarsky *et al.* 2008). However, the presence of large, generalist invertebrate predators in Broadstone could negate this advantage, as leaving the water column to avoid fish predation could make mayflies more vulnerable to attack in the benthos, especially as the large invertebrate predator (*Cordulegaster boltonii* L.) causes significant mortality to mayflies in experimental conditions (see Chapter Two).

Consequently, in Broadstone, there is the potential for two distinct indirect interactions that might influence the success of a non-predatory invader: (a) apparent competition with resident prey species and (b) the effect of behavioural cues from fish on prey vulnerability to benthic predators. Here, I investigate both of these possible processes, using laboratory experiments with a large invertebrate dragonfly predator and varying densities of acid-tolerant and acid-sensitive prey

species, along with a treatment in which chemical cues from fish were present or absent. I hypothesised that:

(a) The acid sensitive species (the putative invader) would suffer a greater rate of mortality from the dragonfly than the acid-tolerant resident (i.e. a necessary – though not alone sufficient - condition for apparent competition)

(b) This difference in survival would be not be modified by the presence of chemical cues from brown trout.

This test of indirect interactions (each involving three species plus cues of a vertebrate predator) can refute the possibility that they can explain, wholly or in part, the slow recovery of benthic communities from acidification. Acceptance of the hypothesis of apparent competition sustains the possibility that it does play a role in nature.

Methods

Study Site

Broadstone Stream (51°05'N 0°02'E) is a spring-fed headwater of the River Medway in SE England, in a poorly buffered catchment of Ashdown Sands (Hildrew 2009). Stream pH varies extensively on an annual basis, from approx. 5.2-6.5 (Layer *et al.* 2011). The system is relatively species-poor, with the predator guild consisting of three large invertebrates (a dragonfly, a caseless caddisfly and an alderfly) and now one vertebrate (*Salmo trutta* L.). The prey community is currently composed mainly of detritivorous stoneflies and chironomid midges, with recent

evidence of sporadic leptophlebiid mayfly population increases (see Chapter One; Townsend *et al* (1983) and Hildrew (2009) for further details).

Laboratory experiments

I selected the largest invertebrate predator, *C.boltonii*, which, in its final instars, is at least one order of magnitude larger, in terms of body mass, than any other invertebrate in the system (Woodward & Hildrew 2001). For the resident species, the most abundant acid-tolerant stonefly, *Nemurella pictetii* Klapálek, was chosen. Both predator and prey have had persistent populations in Broadstone for over a decade (Hildrew *et al.* 1985; Woodward & Hildrew 2001; Layer *et al.* 2011). Additionally, the acid-sensitive species selected was the mayfly *Baetis rhodani* Pictet, from outside of the study reach. However, it has been found very occasionally within Broadstone itself (A.Hildrew, personal communication) and occurs regularly in the lower, circumneutral reaches (Ledger & Hildrew 2001) of Broadstone. *Baetis rhodani* was chosen because it has a pH optimum of 6.06 (Hämäläinen & Huttunen 1996), which makes it likely to invade/recolonize in the near future, given the rate of deacidification in Broadstone over the previous three decades (Woodward *et al.* 2005a; Layer *et al.* 2011). *Baetis* spp. have been widely studied in terms of their ecology and behaviour (Sutcliffe & Carrick 1973; Raddum & Fjellheim 1984; Lepori *et al.* 2003; Tixier *et al.* 2009; Feeley *et al.* 2011), with a documented response to fish chemical cues (Cowan & Peckarsky 1994; Kuhara *et al.* 1999; Peckarsky *et al.* 2008). I used a pH threshold of c. 5.5 to distinguish 'acid-tolerant' species from 'acid-sensitive' species, as it has been specified before to distinguish acidic from circumneutral streams (Hildrew 2009), and is at this point that bicarbonate alkalinity declines to zero and the toxic effects of hydrogen ions and labile aluminium usually become apparent (Sutcliffe & Hildrew 1989; Hämäläinen & Huttunen 1996).*Cordulegaster boltonii* and

N.pictetii were collected from the acidified reaches of Broadstone and *B.rhodani* from the circumneutral, neighbouring Withyham Stream (also in the Medway catchment), from January to June 2013 (see Chapter Two for site details). Predator body size was standardized by selecting only the largest size-class of *C.boltonii* as defined by Woodward & Hildrew (2002a) (i.e. instars 12-14).

I selected densities of prey species following a replacement series design (De Wit 1960), which is widely used by plant ecologists to investigate apparent competition in place of more conventional Lotka-Volterra competition equations (e.g. Inouye & Schaffer 1981), but has also been adapted for use in invertebrate competition experiments (Ayala 1971; Mather & Calgiari 1981; Rodríguez 1997). From the sigmoidal functional response curves derived for *C.boltonii* with each prey species in isolation (e.g. see Fig. 3.1 and text in Chapter Two), I selected two absolute densities of prey: four individuals (hereafter 'low density'), at which attack rate is limited by encounter rate, and 16 individuals ('high density'), at which attack rate plateaus and is limited by handling time. Within these two densities, I altered the relative densities of the prey sequentially, with five relative density treatments per absolute density (see Fig. 3.2). This enabled me to test apparent competition, by assessing relative mortality of both prey species, in both the low and high density-dependent phases of a Type III functional response (as exhibited by *C.boltonii*) and determine whether mortality of either species was altered as a result. In addition, I performed all experiments both with and without exposure to trout chemical cues (kairomones) (see Fig. 3.2 for experimental design), which elicit behavioural responses in certain aquatic invertebrates, including *B.rhodani* (e.g. Abjornsson *et al.* 2001) and *Gammarus pulex* L., another potential circumneutral colonist of recovering acid streams (Hargeby & Petersen 1988; Hargeby 1990; Grabowski *et al.* 2007). I collected this kairomone by keeping an adult *S.trutta*, caught in the circumneutral reach of Broadstone Stream, in a 25 litre containment tank containing water from the same reach. After 24

hours, I harvested the water for immediate use (after Layer et al 2011). During this period, trout were fed on live chironomids, as kairomones are a waste product produced as a result of feeding.

The experiments were conducted in plastic aquaria (19.5cm long x 12cm wide x 13.2cm deep) (SAVIC, Belgium), following the protocols of Woodward & Hildrew (2002a). Washed native gravel substratum was added to each aquarium, to a depth of 5cm, with stream water then added to a total depth of 10cm. I maintained water temperature at 10°C and light on a 12 hours dark/12 hours light cycle. Prior to the experiment, dragonflies were kept isolated in individual containers for three days prior to introducing them to the arenas, in order to standardise motivational state. A single dragonfly was introduced to each arena the night before the experimental period began, to allow time for the predators to acclimatise to their environment. Following this, prey were introduced simultaneously the next morning, with no dietary resources present in the aquaria i.e. resources were extremely limited, although both species could still access biofilm on the substrate. In control trials, mortality of both prey species in the absence of the predator was <1%. For the chemical cue treatments, I immediately added 60ml of kairomone-conditioned water (after Layer *et al.* 2011), which is approximately three times the concentration used by Abjornsson *et al.* (2001) to demonstrate measurable suppression of an acid-sensitive invertebrate and is far above the concentration likely to be present in the stream (Layer *et al.* 2011), in order to maximise my chances of stimulating a detectable behavioural response. After 24 hours, each predator was removed. I then sieved and sorted the gravel, and counted the remaining individuals to assess prey mortality. Each relative density treatment was replicated 6 times, giving 120 sampling units in total (see Fig. 3.2).

Statistical analyses

De Wit replacement series experimental designs (de Wit 1960) measure the competitive ability of the test species by calculation of a coefficient - the 'relative crowding coefficient' (*k*). I calculated this for each species separately using the following formulae (after Williams & McCarthy 2001):

$$k_{n,b} = \left[\frac{1 - p_n}{p_n} \right] x \left[\frac{S_{n,b}}{S_n - S_{n,b}} \right]$$

$$k_{b,n} = \left[\frac{1 - p_b}{P_b} \right] x \left[\frac{S_{b,n}}{S_b - S_{b,n}} \right]$$

Where $k_{n,b}$ = relative crowding coefficient of *N.pictetii*, $k_{b,n}$ = relative crowding coefficient of *B.rhodani*, p_n = proportion of *N.pictetii* in treatment, p_b = proportion of *B.rhodani* in treatment, S_n = survival of *N.pictetii* in monoculture (i.e. solely *N.pictetii* at the absolute density treatment), S_b = survival of *B.rhodani* in monoculture (i.e. solely *B.rhodani* at the absolute density treatment), $S_{n,b}$ = survival of *N.pictetii* in presence of *B.rhodani* and $S_{b,n}$ = survival of *B.rhodani* in presence of *N.pictetii*.

I calculated *k* for each combination of treatments (low absolute density, high absolute density, kairomone absent and kairomone present). If $k_{n,b}$ is equal to one, it indicates that *N.pictetii* is subject to equal amounts of intraspecific competition from itself and interspecific (i.e. apparent) competition from *B.rhodani*. If $k_{n,b}$ is greater than one, then *N.pictetii* is subject to more competition from itself than *B.rhodani*, and consequently if $k_{n,b}$ is less than one, then apparent competitive interactions are more significant than intraspecific interactions (Fransen *et al.* 2001; Williams & McCarthy 2001), and vice-versa for $k_{b,n}$. One-sample *t*-tests indicated if *k* values for all treatment combinations were significantly different from one, which would in turn indicate significant competitive interactions.

I also used input-output ratio diagrams (Futuyma 1970; Ayala 1971; Inouye & Schaffer 1981) to test if the populations of both prey species could maintain a stable co-existence . To do this, I plotted log ratios of *N.pictetii* to *B.rhodani* initial populations (hereafter 'input') against log ratios of *N.pictetii* to *B.rhodani* survival ('output'). If the output ratios are above the 1:1 line on the resulting plot, then it suggests *N.pictetii* will outcompete *B.rhodani*, and vice-versa for below the 1:1 line. If the output ratios intersect the 1:1 line, it suggests there is there is the possibility that populations can co-exist. If the slope of the line which intersects is less than one, it suggests this co-existence is stable and both populations have the potential to persist, and if it is greater than one, it suggests is the populations are unstable and it is unlikely that persistence will occur (Inouye & Schaffer 1981).

In addition, I analysed the effect of absolute density and trout kairomone on both prey species using a fully factorial three-way ANOVA with multiple interaction effects. All statistical analyses were performed using the statistical programming language R (R Development Team 2012)

Results

Comparison of mortalities of the acid-tolerant stonefly and the acid-sensitive mayfly revealed a difference across densities. At the absolute low density treatment, there was evidence that intraspecific competition was occurring among *N.pictetii* individuals, which was shown by *k* values significantly higher than one (*t*-test, $P < 0.05$, see Table 3.1): i.e., there was no evidence of apparent competition between species. However, at the absolute high density treatment, there was evidence of interspecific competition, as *B.rhodani k* values were significantly lower than one (*t*-test, $P < 0.05$), while *N.pictetii k* values were non-significant (see Table 3.1). This indicated that apparent competition occurred between the acid-tolerant and acid-sensitive species once densities were sufficiently high (see Fig. 3.3).

At these high absolute densities, the acid-tolerant species was significantly less vulnerable to dragonfly predation than the acid-sensitive species (in terms of reduced mortality), suggesting that the interspecific competition was asymmetric, increasing the vulnerability of the mayfly. This can be observed on the ratio diagrams (see Fig. 3.4), which show that *B.rhodani* suffered higher mortality in the presence of *N.pictetii* at high densities, whereas at low densities there was essentially a stable co-existence between the two different species – the output ratio line crossed the 1:1 line with a slope of 0.498. This suggests at low absolute densities, these prey species had a dynamically stable co-existence, but as density increased to a higher absolute level, this co-existence became unstable and, as a result of apparent competitive forces, there was potential for *B.rhodani* to be outcompeted. This was shown by the output ratios at high absolute densities being above the 1:1 line with no intersection (see Fig.4).

This pattern, of potential stable co-existence between prey at lower densities and interspecific apparent competition at higher densities, was not altered by the addition of trout kairomones.

Ratio diagrams revealed that with kairomone addition, the output ratio line crossed the 1:1 line at low densities with a slope of 0.908, which indicates stable equilibrium for both treatments i.e. with and without kairomone (see Fig. 3.4). Again, the output ratios at high densities were above the 1:1 line, with no intersection, which is also consistent between treatments. In addition, the *k*-values followed the same pattern for both treatments (i.e. significantly higher than 1 for *N.pictetii* at the low densities, and significantly lower than one at the high densities for *B.rhodani*), indicating that intraspecific competition among acid-tolerant individuals, and interspecific competition between both species at high densities, were also not altered by the addition of trout kairomones (see Table 3.1).

Lastly, ANOVA revealed that the addition of kairomones did not have any effect on the survival of either prey species ($F_{1,24} = 0.05$, $P = 0.83$) (see Table 3.2). This suggests that no behavioural response was stimulated in either the acid-tolerant or acid-sensitive species. However, there was a significant two-way interaction between prey and density ($F_{1,24} = 10.41$, $P < 0.01$) (see Table 3.2), suggesting the competitive dynamics between the acid-tolerant and acid-sensitive prey species shifted between absolute density treatments. This supports the evidence provided by the ratio diagrams, which showed a shift from stability to potentially destabilising interactions as density increased.

Tables & Figures

Table 3.1. The average relative crowding coefficients (*k*) for *N.pictetii* and *B.rhodani* at low (four individuals) and high (16 individuals) absolute densities, and in the presence of absence of trout kairomones, tested using one-sample *t*-tests for significant difference from unity. * denotes $P < 0.05$, ^{ns} denotes $P > 0.05$.

Prey	Kairomone	Absolute Density	Average *k*
Nemurella pictetii	Absent	Low	1.72*
Baetis rhodani	Absent	Low	1.51^{ns}
Nemurella pictetii	Absent	High	1.03^{ns}
Baetis rhodani	Absent	High	0.57*
Nemurella pictetii	Present	Low	1.59^{*}
Baetis rhodani	Present	Low	1.17^{ns}
Nemurella pictetii	Present	High	1.08^{ns}
Baetis rhodani	Present	High	0.56*

Table 3.2. Three-way analysis of variance (ANOVA) testing for effects of prey species, density and trout kairomone on survival rates of prey.

	d.f	**F-ratio**	**P**
Prey	1	11.76	<0.01
Density	1	37.32	<0.001
Kairomone	1	0.12	0.73
Prey x Density	1	10.41	<0.01
Prey x Kairomone	1	0.05	0.83
Density x Kairomone	1	0.15	0.70
Prey x Density x Kairomone	1	0.03	0.86
Error	**24**		
Total	**25**		

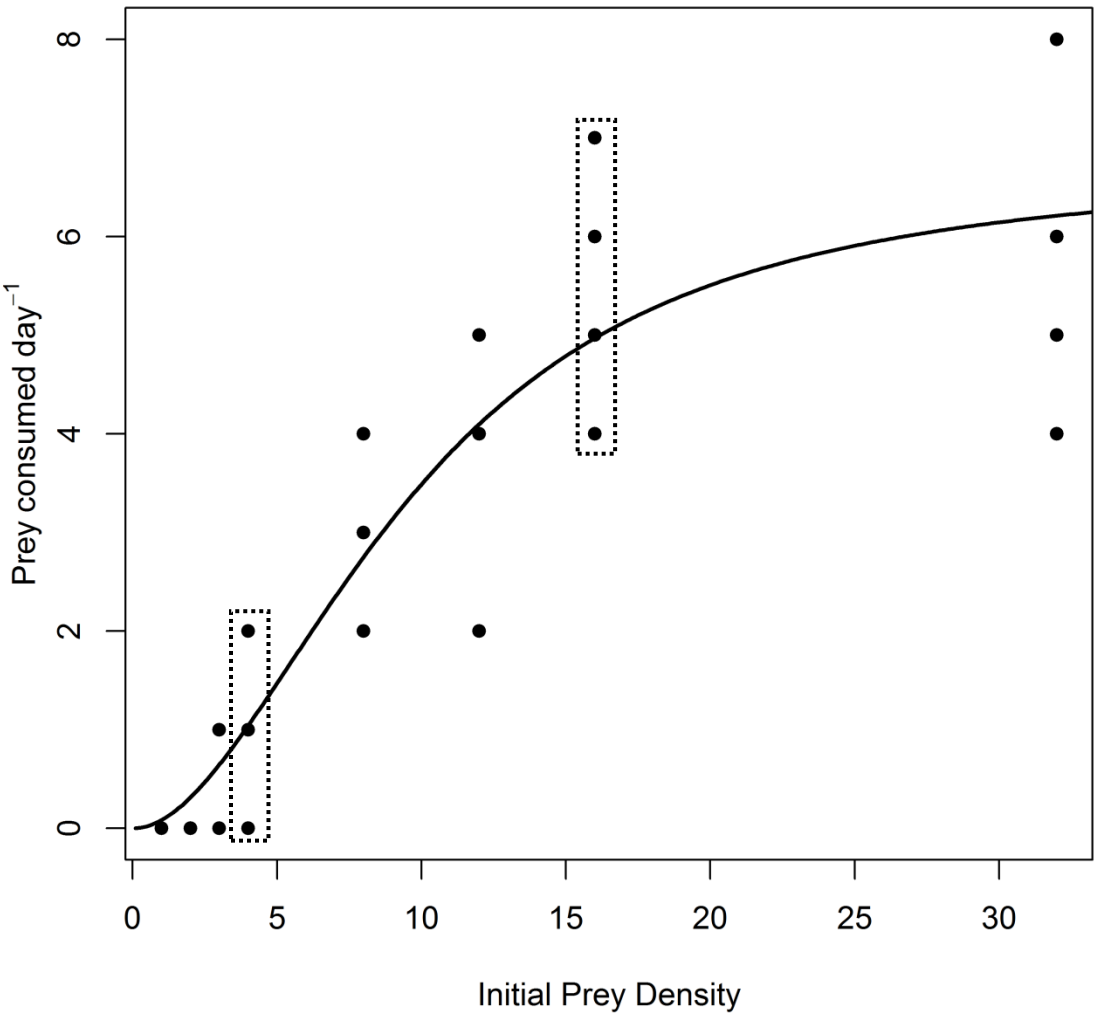

Figure 3.1. Functional response curve of *Cordulegaster boltonii* (predator) – *Baetis rhodani* (prey) interaction, at 10°C, with initial prey density on the x-axis and the number of prey eaten on the y-axis. Highlighted with dotted lines are the experimental 'low' (four prey individuals) and 'high' (16 prey individuals) densities.

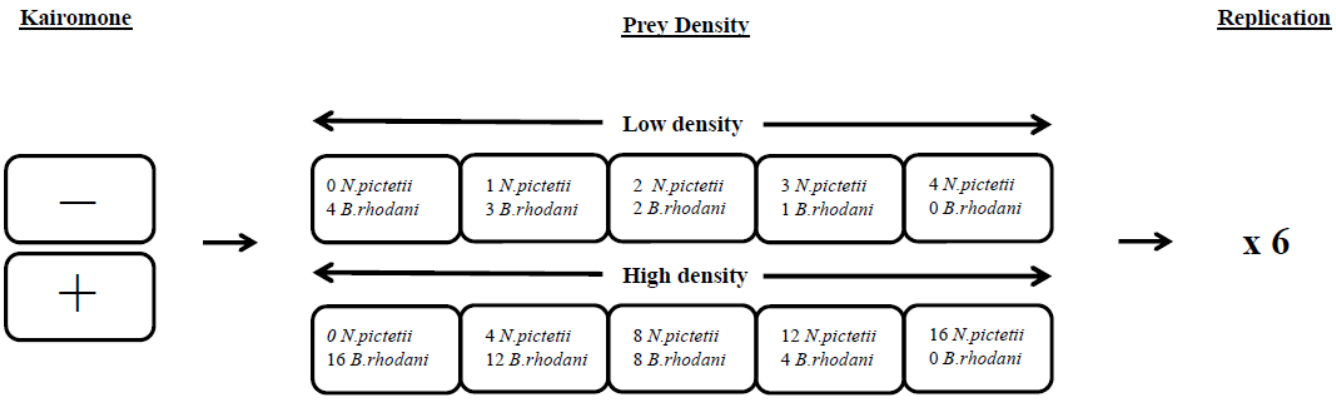

Figure 3.2. Experimental design for indirect interaction study. Total *n* =120.

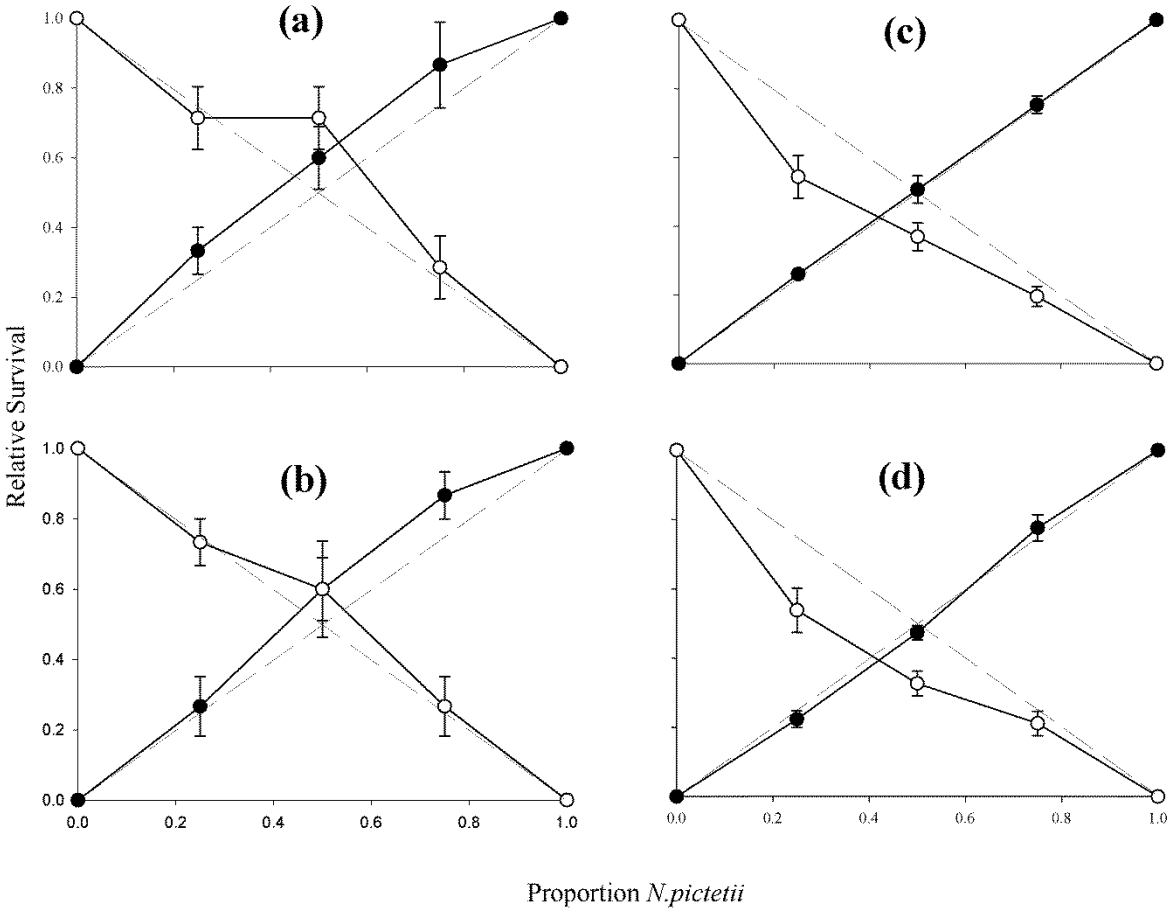

Figure 3.3. De Wit replacement diagrams of proportion of *N.pictetii* in test aquaria against relative survival of each prey species for (a) – Low absolute density, kairomone absent (b) – low absolute density, kairomone present (c) – High absolute density, kairomone absent (d) – High absolute density, kairomone present. Black circles represent *N.pictetii*, white circles represent *B.rhodani*. Dotted lines are reference values, representing equal intra- and interspecific competition i.e. $k = 1$.

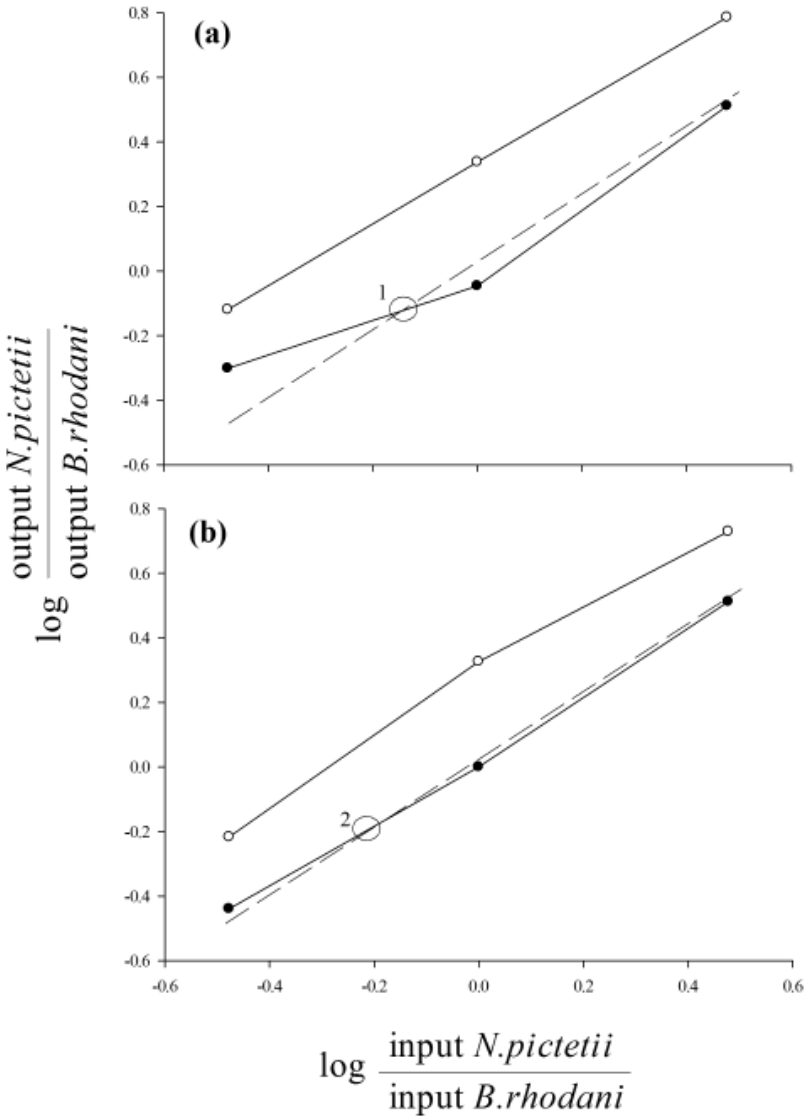

Figure 3.4. Ratio diagrams showing relationship between log input and output ratios of the two prey species for (a) kairomone absent treatment and (b) kairomone present treatment. Black circles represent low absolute density (four individuals per aquaria) and white circles represent high absolute density (16 individuals per aquaria). Dotted lines represent 1:1 line i.e. $k = 1$. Low absolute density line crosses 1:1 line with a slope of 0.468 (highlighted in Panel (a) as 1) for the kairomone absent treatment and with a slope of 0.908 (highlighted in Panel (b) as 2) for the kairomone present treatment. In both cases this indicates a stable co-existence between prey species.

Discussion

The occurrence of lags, or hystereses, in biological recovery following chemical amelioration of freshwater acidification (UKAWMN 2010) is yet to be fully understood. It is possible that indirect interactions, in the form of apparent competition, within communities are at least partially responsible, given that the mechanisms that govern competing species within natural systems are a crucial area in our understanding of community ecology (Begon *et al.* 2009).

Here, I observed strong evidence of indirect apparent competition between *B.rhodani* and *N.pictetii* at high absolute population densities, which is broadly consistent with my first hypothesis that apparent competition would result in reduced survival of the acid-sensitive species. Predation on the acid-tolerant prey species was markedly lower, as hypothesised, and both the *k* coefficients and input/output ratios suggest that the acid-sensitive prey suffer stronger predation and hence stronger apparent competition than the acid-tolerant species. As there were extremely limited shared resources in the experimental arenas, it is likely that apparent competitive interactions are driving this outcome. This competitive exclusion was not universal, with low absolute density treatments suggesting a stable coexistence between species, as opposed to the exclusion of either prey group. Indeed, from the *k* coefficient values, it appears that the primary competitive interactions in acid-tolerant species at low densities are intraspecific, with potentially no indirect competition between acid-tolerant and acid-sensitive species.

This shift from a potentially stable co-existence at low absolute densities (see Fig. 3.3) to strong apparent competition, leading to reduced survival and possibly exclusion of the acid-sensitive species, at high absolute densities was clearly density-dependent. Essentially, at low population density there is scope for co-existence, but as densities increase, the availability of 'enemy-free space' (Holt 1977) is likely to decrease. As a result, the likelihood is that various mechanisms of

apparent competition will act on the prey population. At high densities (in this three-species network) the predators are being supported by two separate prey species, and encounter rates are likely to increase. It can therefore exert a strong top-down pressure, as shown here, on the acid-sensitive species without detrimental effects on its own survival, as the acid-tolerant prey provide an alternate resource. As encounter rate is a significant driver of predation in species-poor systems, such as Broadstone (Woodward *et al.* 2005a; Hildrew 2009), this increase in predation pressure is likely to lead to increased mortality in the more vulnerable, acid-sensitive prey assemblage.

The indirect interactions observed here, between acid-sensitive and acid-tolerant prey species, could potentially result in a disproportionate level of mortality in the more mobile mayfly population (Peckarsky 1996), which could in turn result in an inability for these populations to persist at high densities in the presence of large invertebrate predators. As well as this experimental evidence, there is a suggestion this mechanism may already be operating from existing survey data within Broadstone. *Paraleptophlebia submarginata*, an acid-sensitive leptophlebiid mayfly, was first detected in the system in the mid-1990s (Woodward & Hildrew 2001); initially sporadically, yet it now persists in the system, but only at extremely low population densities, and has done so for over a decade (Woodward *et al.* 2005a; Layer *et al.* 2011). Apparent competition between this novel species and the resident prey assemblage for 'enemy-free space' could well be responsible for the low density of *P.submarginata*, despite apparently favourable physicochemical conditions (*P.submarginata* has a pH optimum of 5.6 (Hämäläinen & Huttunen 1996), which is also the average annual pH of Broadstone Stream (Layer *et al.* 2011)).

The suggestion that species interactions contribute to a lag in biological recovery has already been explored in the context of acidification, via inference from survey data and mathematical simulations of food web dynamics (Layer et al 2011). Hildrew & Ledger (2005) suggested that

the ‘community closure’ hypothesis (Lundberg *et al.* 2000) can be applied to recovering acid streams, with the functional feeding characteristics of acid-tolerant species expanding to encompass the algal resources of acid-sensitive grazers, once physicochemical conditions exclude the latter. Once chemical conditions ameliorate, however, there is no corresponding suggestion that such acid-tolerant species are excluded (Monteith *et al.* 2005; Hildrew & Ledger 2005) or impaired (Lancaster 1988; Ledger & Hildrew 2001) by increasing pH alone. This could result in alterations in both the structure and functioning of the web, with the dietary breadth of generalist detritivores a key factor in resistance to colonisation (Hildrew & Ledger 2005). The likelihood of indirect competitive interactions between acid-tolerant and acid-sensitive species, as demonstrated here, further strengthens the idea of the acid-tolerant community resisting the recolonisation of acid-sensitive species and potentially explains, at least partially, why biological recovery has not been observed on a large scale in acidified fresh waters.

However, biological changes have been apparent in Broadstone Stream – not just in the sporadic occurrences at low densities of primary consumers such as *P.submarginata*, but in marked successive invasions, or irruptions, at the top of the food web (Hildrew *et al.* 1985; Woodward *et al.* 2005a; Layer *et al.* 2011). In the 1980s, 1990s and 2000s, the dominant large predator has, on each occasion, been superseded by a progressively larger predator, culminating in the invasion of brown trout (Layer *et al.* 2011). Such changes at the top of the web, but not lower down, were not anticipated *a priori* however. Clearly, deacidification does bring ecological changes, but not what was expected to be a simple replacement of one set of species by another. The ultimate trajectory of such changes will be of great interest as the environment continues to change, and not just in acidity.

It has become increasingly evident that body size plays a significant role in the organisation of ecological networks (Williams & Martinez 2000; Jonsson *et al.* 2005; Woodward *et al* 2005b), and the impact of size could be key in Broadstone Stream. If each successively larger predator

occupies the apex of the food web in turn, then that species effectively gains 'size refugia' from predation. Each new predator irruption increases the scope for apparent competition, as smaller predators' diets are subsets of those of the next largest, which could result in a general increase in top-down control of the prey assemblage (Layer *et al.* 2011). Although these successively larger predators are vulnerable to mutual predation at early stages in their life cycles via ontogenetic switches, it is likely than on average there is little direct predation pressure to mediate against population expansion and subsequent persistence of these species.

However, the presence of these predators could actually have indirect benefits for the resident, acid tolerant prey assemblage. Mayflies (and gammarid shrimps) can reach very high numbers in streams containing voracious drift-feeding predators (mainly salmonids), than without, presumably as a result of using adaptive behavioural cues enabling them to reduce predation risk from that source (Peckarsky *et al.* 2008). The presence of fish might then allow such acid-sensitive prey to modulate indirect competition from resident species and to establish themselves through mediated predation pressure. However, consistent with my second hypothesis, that there will be no behaviourally mediated effect of trout cues, I found no evidence of fish kairomones eliciting a behavioural response in the acid-sensitive mayflies, in contrast to other studies (e.g. Cowan & Peckarsky 1994; Kahura *et al.* 1999; Abjornsson *et al.* 2001). This suggests that successive invasions of large predators may be more likely to impact on the prey assemblage via direct consumption. Woodward & Hildrew (2002a) noted that the highly cryptic nature of *C.boltonii*, combined with its rapid feeding response (Pritchard 1965), could negate avoidance in resident Broadstone taxa, a factor which could also explain the lack of behavioural avoidance shown here.

What this ultimately might suggest is that aquatic communities do not simply have vacant ecological niches to which previously excluded species can simply return once toxicological restrictions have eased, and that there is likely sufficient direct and indirect pressure on invasive acid-sensitive prey, from multiple trophic levels, to resist persistence of novel colonisers. Rather, through varying mechanisms at several 'levels of organisation', including that of the network as here, the acid-tolerant community could be inherently stable and robust (Layer *et al.* 2010a; 2013). Essentially, this suggests that, at least in the transient phase, biological recovery from acidification will not follow a simple reversal of the decline as favourable chemical conditions appear, but that recovery could be non-linear, and that more complex and subtle ecological factors govern community stability and structure.

Chapter Four: Effects of long-term amelioration of acidification and invasion by a vertebrate predator on a well-characterised food web

This chapter contributed to the publication of: Layer K, Hildrew AG, **Jenkins GB**, Riede JO, Rossiter SJ, Townsend CR, Woodward G (2011) Long term dynamics of a well-characterised food web: Four decades of acidification and recovery in the Broadstone Stream model system, *Advances in Ecological Research,* **44**, 69-117.

Abstract

The ecology of Broadstone Stream, a recovering acid stream, has been studied intensively for over four decades, and the previously invertebrate-dominated food web is one of the best characterised of any natural system. Size-structured scaling is evident among the feeding hierarchy of the macroinvertebrate predator guild, while the food web is highly reticulate and seemingly dynamically stable. Although many freshwater systems, including Broadstone, have recovered chemically from the acidification in the 20th century, widespread biological recovery still lags behind. Broadstone, however, has seen irruptions of successively larger-bodied predators over the monitoring period, cumulating in the mid-2000s, when brown trout (*Salmo trutta* L.) were detected for the first time. This new vertebrate predator was previously excluded due to the acidity of the stream. It potentially represents a significant perturbation, in that it is several orders of magnitude larger than any members of the existing predator guild. Here I present evidence, using existing decadal-scale data combined with novel experimental manipulations, that *S.trutta* has the

potential to alter the structure and stability of the resident community through direct top-down predation pressure, while seemingly inducing no behavioural indirect responses from the resident acid-tolerant community. Inferred interaction strengths and food chain lengths have increased, while species abundance has decreased. Through exploration of the long-term dataset, it was also shown that these trends in community structure can be observed over waves of successive invertebrate predator population irruptions in the system, allowing inferences to be made on the role of internal dynamics in recovering acidic freshwater systems.

Introduction

Knowledge of the effects of and responses to both biotic (e.g. invasions) and abiotic (e.g. acidification) perturbations on natural ecosystems is important if community structure and ecosystem processes, and their interdependence, are to be fully understood. The invasion of a novel species, especially one which represents a dramatic shift in body size within the community, can have impacts that range from modest, transient changes in individual species abundance and distribution, to widespread species extinctions and extensive 'rewiring' of the food web, which can alter the stability of the whole system (O'Gorman & Emmerson 2009; Montoya *et al.* 2009). The effect of such perturbations is connected to both complexity, in terms of the number of species and their trophic links, and interactions strength and how these might confer resilience and resistance upon the food web. The study of ecological networks in general, and food webs in particular, is therefore crucial for understanding community dynamics under environmental change (Ings *et al.* 2009).

The anthropogenic acidification of fresh waters, its consequences and subsequent amelioration have been of great scientific interest for over 60 years (e.g. Gorham 1955; 1998; Flower & Battarbee 1983; Layer *et al.* 2010a,b; 2011). The ecological effects on fresh waters of increasing industrialisation through the 19th and 20th centuries were profound, with significant increases in acid deposition leading to large-scale loss of many acid-sensitive species and of economically-important salmonid stocks (e.g. Atlantic salmon (*Salmo salar* L.), brown trout, Arctic charr (*Salvelinus alpinus* L.), which attracted substantial public attention (Rosemund *et al.* 1992; Hildrew & Ormerod 1995). Since the diagnosis of the causes of acidification and subsequent legislation against polluting emissions, there has been a noticeable increase in the pH of rainfall and sensitive surface waters, as well as other signs of chemical recovery, across both Europe (Monteith *et al.* 2005; UKAWMN 2010) and North America (Stoddard *et al.* 1999; Driscoll *et al.* 2007). However, the biological response of affected communities has not been so evident. Several hypotheses have been put forward to explain why that may be, including repeated persistent acid episodes, dispersal limitations on returning acid-sensitive species and intrinsic food web resistance to re-colonisation (see Chapter One for details). This chapter focusses on the impact that successful biological recovery, in the form of species recolonisation, can have on a network.

Broadstone Stream, a formerly acid (and acidified) system now recovering, is one of the best-characterised freshwater systems in the world, with the food web having been studied since the 1970s (e.g. Hildrew & Townsend 1976 ; Woodward *et al.* 2005a; Hildrew 2009; Oleson *et al.* 2010). It is therefore an ideal model system with which to observe the potential impacts of species invasions in response to chemical recovery from acidification (Hildrew 2009). It is strongly size-structured – a property shared with many other aquatic systems (e.g. Cohen *et al.* 2003; Hildrew *et al.* 2007; O'Gorman *et al.* 2010) – and the top trophic level was dominated by a guild of predatory macroinvertebrates from at least the early 1970s until the turn of the

millennium. Amelioration of acidity was associated with an irruption in the 1990s of a formerly extremely scarce large-bodied invertebrate predator, the dragonfly *Cordulegaster boltonii* Donovan (Woodward & Hildrew 2001). In the early years of the new century, however, a vertebrate predator (brown trout) was detected for the first time, representing an increase of several orders of magnitude in the size of the apex predator.

Body size has long been recognised as a significant factor in food web dynamics and stability (e.g. Elton 1927; Peters 1983; Jennings & Mackinson 2003; Emmerson & Rafaelli 2004; Woodward *et al.* 2005b; Hildrew *et al.* 2007) and, consequently, the significant shift in size distribution represented by the trout invasion of Broadstone Stream has potential consequences for a whole suite of key food web properties. Mass-abundance relationships can have a strong impact food web topology (Cohen *et al.* 2003). On a species-averaged scale, allometric scaling can be useful in establishing generalities across ecosystems (Woodward *et al.* 2005b), but such mass-abundance data must be averaged to be analysed, due to data being collected on a taxonomic basis, which results in large variations in body masses. As such, this approach leads to a loss in resolution. An alternative is to view mass-abundance relationships on an individual basis, regardless of species identity, which can then be used to provide more precise information about processes, such as energy flux through the system (e.g. Woodward & Warren 2007; Yvon-Durocher *et al.* 2011).

Using both these metrics of mass-abundance relationships can be used to analyse shifts in food web structure in response to a perturbation; in this case the invasion of a novel predator. These alterations in structure, combined with the natural history of the brown trout as a voracious, generalist and versatile predator (Bagliniere & Maisse 1999), can dramatically alter the structure and stability of the food web (Layer et al 2010b), through increasing food chain length, body size

ratios and, consequently, interaction strength. Assessing the body size of consumers and resources, and the ratio between them, is an effective way of inferring interaction strength (Emmerson & Rafaelli 2004). Strong interactions are often seen as having a negative effect on the dynamic stability of a network (McCann 2000; O'Gorman & Emmerson 2009) and, given that trout often exert strong top-down pressure on prey (Flecker & Townsend 1994; Nakano *et al.* 1999; Townsend & Simon 2006), it could be expected that this direct effect of the trout invasion will have a noticeable effect.

There are also potentially further, and more subtle, indirect consequences of the trout invasion. Predators can, and often do, have both direct & indirect interactions throughout food webs (Knight *et al.* 2005). The role of behaviour is increasingly appreciated in studies of ecological networks (Petchey *et al.* 2008; Ings *et al.* 2009; Brose 2010; Kefi *et al.* 2012), and the mere presence of a novel dominant predator can alter prey dynamics, even in the absence of direct consumption (Sih *et al.* 1998; Priesser *et al.* 2005). There are various cues that can affect prey behaviour (e.g. visual, hydrochemical, auditory), but here I focus primarily on chemical cues produced by fish, known as kairomones, which can induce defensive invertebrate behavioural response in freshwater animals (Sih 1986; McIntosh & Peckarsky 1996; Abjornsson *et al.* 2001). The largest invertebrates in Broadstone, the predator guild, are predominantly sedentary, ambush predators (Woodward & Hildrew 2001). Consequently, it is likely that visual cues will elicit no measurable response in these taxa, given that relative inactivity is a normal characteristic rather than a behavioural response. However, one abundant large invertebrate predator in the system (*Sialis fuliginosa* Pictet) is a more active hunting predator which might respond to chemical signals from fish (Chivers *et al.* 1996), most obviously by decreased mobility thus avoiding detection. Additionally, smaller invertebrates, such as the primary consumers that are eaten by the larger invertebrate predators, have the potential to be affected by behavioural cues. If so, this

alteration in prey activity has important consequences for encounter rate with the invertebrate predators, as well as with trout. Encounter rate is the key determinant of predation rate by invertebrate predators in Broadstone Stream, given the scarcity of prey (Woodward & Hildrew 2002b). Consequently, if prey behaviour is altered by the presence of trout, it could lead to a potential weakening in the strength of interactions between invertebrate predators and their prey, which in turn could lead to a 'behaviourally-mediated cascade'; similar in nature to an antagonistic trophic cascade (Beckerman *et al.* 1997; Schmitz *et al.* 1997), albeit at different levels of organisation.

In this Chapter, I examine the impact of brown trout on the structure of the Broadstone Stream food web, by investigating whether the invasion is likely to have changed the structure of the food web. To do this, I assessed both species-averaged and individually-distributed patterns in mass-abundance over four decades, to analyse whether these different metrics show a shift in mass-abundance scaling in response to trout invasion. I also used inferred interaction strengths, another body size-related metric, to assess if trout are likely to be exerting a 'top-down' direct effect on the community. Additionally, I performed laboratory experiments, using a range of invertebrate predators and prey of differing mobility, to see if this direct antagonistic effect was acting in parallel with a non-trophic indirect effect i.e. if behavioural avoidance was altering predator-prey interactions. If behavioural effects were not evident, it was likely that direct consumption of prey will be the key determinant of the effect of trout on the system, and any consequent alteration in food web structure and function. Previous experiments using Broadstone taxa (Townsend & Hildrew 1977; Woodward & Hildrew 2002c) suggest that direct effects of predators are more important within the system, and I expected this to be the case in this study.

I hypothesised that: (a) brown trout invasion will have a significant impact on the structure and function of the Broadstone food web (via an increase in mean food chain length and interaction strengths determined by body size) and (b) this impact will be a result of direct, top-down trophic effects as opposed to non-trophic behavioural effects.

Methods

Study site

Broadstone Stream (51°05'N 0°02'E) is a spring-fed headwater of the River Medway in SE England, within a catchment of soft sandstone (Ashdown Sands), which provides little buffering capacity (Hildrew 2009). The catchment has predominantly heath and woodland vegetation. Stream pH typically ranges from about 5.2-6.5 seasonally, with the annual peak occurring in summer and the minimum in winter. There has, however, been a general and progressive decline in acidity since the 1970s, and particularly so in the summer months when most taxa are in their most acid-sensitive early life-stages (Woodward *et al.* 2002), and this long-term rise in pH has been observed in several other neighbouring streams in the Ashdown Forest (Hildrew 2009; Jenkins *et al.* 2013). The apex predator in the system is now the brown trout. The macroinvertebrate community of Broadstone is species-poor and dominated by acid-tolerant insect larvae, with an abundance of invertebrate predators, the largest of which is the golden-ringed dragonfly (*C. boltonii*), whose population in stream irrupted in the 1990s. The two other dominant large macroinvertebrate predators are the larvae of the alderfly *Sialis fuliginosa* Pictet, which was most abundant in the 1980s, and the net-spinning caddis *Plectrocnemia conspersa*

Curtis, which was the dominant predator when the system was first studied in the 1970s. The macroinvertebrate prey assemblage is composed mainly of detritivorous stoneflies and chironomid midges, with recent evidence of an increase in leptophlebiid mayflies (see Table 4.1 for a list of the main taxa, and Hildrew (2009) for a full list), which in turn reflects the dominance of allochthonous detritus, especially coarse particulate organic matter (CPOM) in the form of terrestrial leaf-litter, as the main basal resource. Algal resources are scarce, due to a combination of the stream's acidity and the dense shade cast by the riparian canopy.

Characterising and constructing the food webs

Broadstone Stream has been regularly sampled since the 1970s and the data used in this chapter were collected in 1976 (CR Townsend, AG Hildrew), 1984 (K Schofield, J Lancaster), 1997 (G Woodward) & 2006 (K Layer) using comparable methods. The absolute abundance of macroinvertebrates were quantified by taking Surber samples (sample-unit area 0.0625m^2; mesh aperture 330μm, after Townsend & Hildrew 1983) from the same 200m-stretch on each sampling occasion. Samples were preserved in 70% Industrial Methylated Spirit. In the laboratory, macroinvertebrates were sorted from debris, identified to species where possible (except dipteran larvae) and counted. Head capsule width or body length of each individual was measured using a calibrated ocular micrometer (x200 to x400 magnification) and used to derive individual body mass estimates from published regression equations (Woodward *et al.* 2005a). Trout abundance was quantified in 2006 (Layer 2010) by depletion electrofishing within a 100m stretch of the study reach (after Junge & Libosvarsky 1965). Stop-nets were installed at each end of the stretch, and three runs were completed with a Smith-Root LR-24 backpack electrofisher, moving upstream and sweeping from one side of the stream to the other. The number of fish caught and

the fork-length and body mass of each individual were recorded, prior to release into the stream after the third run.

I compared sampling decades by standardising taxonomic resolution to the lowest common denominator among samples (after Woodward *et al.* (2002)) e.g. all Chironomidae species were grouped into either the predatory 'Tanypodinae' or 'non-predatory Chironomidae'. This meant that network size, in terms of the absolute number of nodes and links, was smaller than in the most highly-resolved versions of the food web (e.g. Woodward *et al.* 2005a), but this standardisation was necessary for making meaningful comparisons across the four decades. I excluded terrestrial prey from all the food webs, because they included a wide range of often unidentified taxa with unknown body masses. I then used this data to construct "animal only" food webs (i.e. (excluding meio- and microfauna) across the four decades, and then carried out formal statistical analyses using only data collected for comparable months, within a single annual cycle per decade, in order to match the recruitment-oviposition cycle as closely as possible. The trophic networks were plotted as trivariate food webs on $\log_{10}$ (mean individual body mass)-$\log_{10}$ (abundance) scatterplots (*sensu* Cohen *et al.* 2003). The strength of feeding links was also calculated, by using the species-averaged body mass ratios of predator and prey, raised to the ¾ power as a proxy measure of interaction strength (Emmerson *et al.* 2005; Berlow *et al*. 2009; Layer *et al*. 2010b).

Individual-level data on body mass were also available for each taxon within the food web from direct measurements made on >40,000 individuals in the 1970s, 1990s and 2000s (Woodward *et al.* 2005a, 2010; Layer *et al.* 2010b) and these were compiled to calculate species-averaged body masses for each decade. Unfortunately, although abundances were recorded no direct measurements of body mass for all species were available for the 1980s. Instead, by applying the

same mean individual mass derived in the 1990s for a given month to the individuals for each taxon for the corresponding month in the 1980s, and then weighting by the observed monthly abundance values (in the 1980s), which were used to hindcast annual means of body mass for each taxon. In addition to species-averaged allometric scaling relationships between mass and abundance and feeding links (e.g. after Cohen *et al.* 2003), Individual Size Distributions (ISDs) were also constructed by assigning each individual organism, irrespective of species identity, to a series of logarithmic size bins (after Layer *et al.* 2010a) to assess potential shifts in size-based allometries over time (e.g. after Brown *et al.* 2004). This comparison between different methodologies allows a focus on the impact of body size, which constrains the life history of many organism (Brown *et al.* 2004). ISDs were constructed for one month only per decade (August) during the height of the oviposition period, when the size range and abundance of new recruits is at its peak for most invertebrate species. ISDs could not be hindcast for the 1980s, unlike the situation for species-averaged allometric data, as the required individual-level data were not available.

Laboratory experiments

I measured survival rates of two detritivorous stoneflies (*N.pictetii* and *L.nigra*), in the presence of each of the three dominant large invertebrate predators (*C.boltonii, S.fuliginosa* and *P.conspersa*) in the presence/absence of *S.trutta* chemical cues (kairomones). I collected invertebrate predators and prey from the acidified section of Broadstone between November and February 2010. Standardisation for size among the predators by only using final instars of *S.fuliginosa* and *P.conspersa*, and the largest size-class of *C.boltonii* (i.e. instars 12-14, after Woodward & Hildrew (2002a)). The experiments were conducted in three litre capacity plastic aquaria (SAVIC,

Belgium), following the protocols of Woodward & Hildrew (2002a). Washed native gravel substratum was added to each aquarium, to a depth of 5cm, with stream water then added to a total depth of 10cm. Stream water was collected from Lone Oak Stream, a neighbouring headwater of the Medway which has almost identical water chemistry to Broadstone, but no fish (see Layer *et al*. 2010b), to eliminate the possibility of resident kairomones being present. Water temperature was maintained at 10°C and the light regime was 12 hours light/12 hours dark. Prior to the experiment, I kept predators in individual containers and starved them for five days, to standardise hunger, and then added an individual predator to the test aquaria the night before the experiment began, to allow individuals to adjust to the environment and especially to enable *P.conspersa* larvae sufficient time to spin nets, as this is their primary means of prey capture (Townsend & Hildrew 1979). I set initial prey density at 10 individuals per aquaria, then added 60ml of kairomone-exposed water – harvested from a containment tank containing one adult *S.trutta*. This resulted in a dosage of approximately 3x the concentration of that which Abjornsson *et al.* (2001) used to demonstrate measurable suppression of activity of *G.pulex* i.e. I used an amount of kairomone far beyond the level likely to be encountered in the stream, to maximise the chances of eliciting and detecting any potential behavioural response. I ran trials for each predator type with/without kairomone addition, with 10 replicates per treatment (i.e. 120 microcosms in total). Each trial was run for 24 hours and then elutriated and sorted the gravel to count all surviving *N.pictetii*/*L.nigra* and establish predation rates, after Woodward and Hildrew (2002a).

Statistical analyses

I constructed trivariate food webs and ISD plots using the "cheddar" package (Hudson *et al.* 2013) for the statistical programing language R (R Development Team 2012). I then calculated log-

transformed, mass-abundance regression slopes from both the trivariate and ISD plots using ordinary least squares regression (OLS) analysis, and then compared the ISD plots using analysis of co-variance (ANCOVA), in order to assess the direction and magnitude of individual size shifts in the food web through time.

To normalise data and enable comparison of predation across treatments, I arcsine-transformed the data and calculated survival using the following formula, after Woodward & Hildrew (2002a). *X* denotes number of prey surviving after 24 hours of predator exposure, and *n* denotes initial prey density:

$$p' = \arcsin\sqrt{\frac{X + \frac{3}{8}}{n + \frac{3}{4}}} \text{ (Zar, 1996)}$$

I then analysed the effect of predator species and trout kairomones on prey mortality for both prey species using fully factorial analysis of variance (ANOVA), with kairomone presence/absence and predator species as main effects in a crossed design. I also assessed frequency distributions of inferred interaction strengths over the four decades using goodness-of-fit χ^2 tests. The direction of these distributions (i.e. assessment of progressive change through time) was analysed using Mann-Whitney pairwise comparisons. These statistical analyses were performed using MINITAB Version 14 (Minitab Inc., Pennsylvania, USA).

Results

Mass-abundance patterns

For both the pre- and post-trout invasion trivariate food webs, the general direction of energy flux was from smaller and more abundant to smaller number of larger and rarer taxa. The regression slope for the 1970s (b = -1.04) suggests an equal biomass density scaling relationship. However, the regression slope became progressively shallower, firstly with the invasion of *C.boltonii* (b = -0.7) in the 1990s and then *S.trutta* (b = -0.4) in the 2000s. The numerical abundance of all species has significantly decreased from the 1970s to 2000s (ANOVA, $F_{2,39} = 17.27$, $P < 0.001$, Bonferroni *post-hoc* comparisons, $P < 0.001$), but individual body masses have not significantly decreased correspondingly (ANOVA, $F_{2,39} = 2.49$, $P > 0.05$). Essentially, the body mass-abundance scaling relationships of the network changed markedly over time, as abundances declined and body mass increased (Fig. 4.1).

The Individual Size Distributions (ISDs) for July-August (i.e. during the peak of invertebrate recruitment) revealed marked shifts in the size structure of the food web, with the logM-logN regression slope being significantly shallower in the 2000s, after the trout invasion, than in the 1970s and 1990s (ANCOVA: $F_{1,25} = 7.80$ $P = 0.01$). Essentially, mean abundance of the smaller members of the food web declined, whilst mean individual body size increased, principally because the new dominant predators occupied existing (or created new) size bins at the upper end of the size spectrum (Fig. 4.2).

Frequency distributions of inferred interaction strengths among consumer-resource pairs showed significant change from the 1970s to 2000s ($\chi^2 = 29.764$ df = 6, $P < 0.001$ (Table 4.2)) with a significant pairwise difference between the 1970s and 1980s (Kruskal-Wallis, $H_3 = 11.36$, $P = 0.01$, 1970s < 2000s Mann-Whitney pairwise comparison ($P = 0.02$)) with an especially pronounced rise after the trout invasion.

Laboratory experiments

Trout kairomones did not alter predation by the three large resident invertebrate predators on their stonefly prey. There were, however, significant differences in predation rate among the predators (*C. boltonii* > *P. conspersa* > *S. fuliginosa*), with the largest (*C. boltonii)* exerting far stronger predation pressure than the two smaller predators (Table 4.3; Fig. 4.3). The lack of a kairomone main effect or a significant interaction term revealed that chemical cues did not alter prey (or predator) behaviour sufficiently to affect direct predatory feeding links.

Tables & Figures

Table 4.1. The Broadstone Stream food web taxa (1970s – 2000s)

	Taxon
Apex predator	*Salmo trutta* L. (Salmoniformes: Salmonidae)
Predators	*Cordulegaster boltonii* Donovan (Anisoptera: Cordulegasteridae); *Sialis fuliginosa* Pictet (Megaloptera: Sialidae); *Siphonoperla torrentium* Pictet (Plecoptera: Chloroperlidae); *Plectrocnemia conspersa* Curtis (Trichoptera: Polycentropodidae); Tanypodinae.
Primary consumers	*Nemouridae*; *Leuctra hippopus* Kempny (Plecoptera: Lecutridae); *Leuctra nigra* Olivier (Plecoptera: Leuctridae); *Halesus radiatus* Curtis (Trichoptera: Limnephilidae); *Potamophylax cingulatus* Stephens (Trichoptera: Limnephilidae); *Asellus meridianus* L. (Isoperla: Asellidae); *Niphargus aquilex* Schiödte (Amphipoda: Niphargidae); *Paraleptophlebia submarginata* Stephens (Ephemeroptera: Leptophlebidae); *Pisidium* sp. (Veneroida: Spheriidae); Tipulidae; Chironomidae (non-predatory); Coleoptera; Simuliidae.

Table 4.2. Chi-squared goodness of fit test for three classes of interaction strength (Weak (-2 to 0), Moderate (0 to 2) and Strong (2+)) over four decades in Broadstone Stream.

Interaction Strength		**1970s**	**1980s**	**1990s**	**2000s**	**Total**
Weak	Observed	15	21	26	19	81
	Expected	14.16	15.86	24.36	26.62	
	χ^2	0.05	1.666	0.111	2.182	
Moderate	Observed	10	7	17	16	50
	Expected	8.74	9.79	15.03	16.143	
	χ^2	0.181	0.795	0.257	0.011	
Strong	Observed	0	0	0	12	12
	Expected	2.10	2.35	3.61	3.94	
	χ^2	2.098	2.35	3.605	16.455	
Total		25	28	43	47	143

Table 4.3. Fully factorial ANOVA testing for effects of predator species identity and presence/absence of fish kairomone on prey survival rates in laboratory experiment (n = 10 replicates per treatment, 120 aquaria in total).

	d.f	**F-ratio**	**P**
Predator	2	13.06	<0.001
Prey	1	1.71	0.193
Kairomone	1	0.27	0.606
Predator x prey	2	6.76	0.002
Prey x kairomone	1	0.03	0.857
Predator x kairomone	2	1.22	0.298
Predator x kairomone x prey	2	2.32	0.103

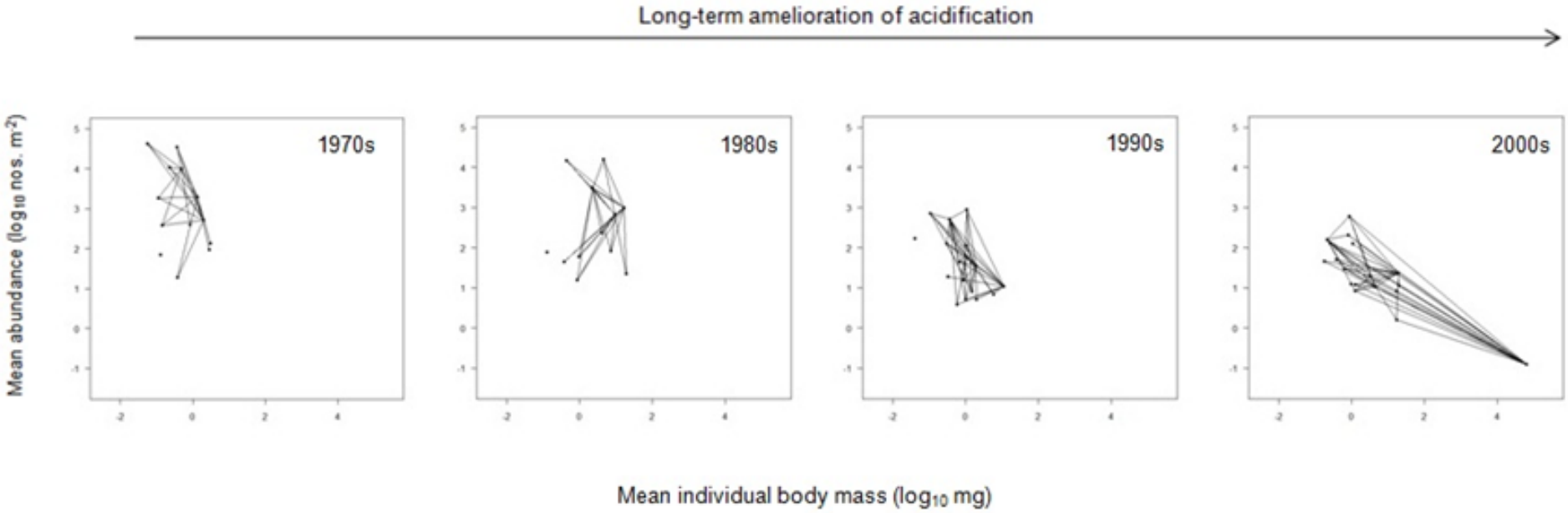

Figure 4.1. The pre- and post-invasion Broadstone Stream trivariate food webs depicted using species-averaged data of $\log_{10}$-transformed abundance-body mass relationships and food web structure. Regression equations as follows: (1970s) $y=-1.04x + 2.95$, $F = 7.05$, $P < 0.05$ (1990s) $y=-0.7x + 1.71$, $F = 5.77$, $P < 0.05$ (2000s) $y=-0.54x + 1.6$, $F = 5.77$, $P < 0.001$.

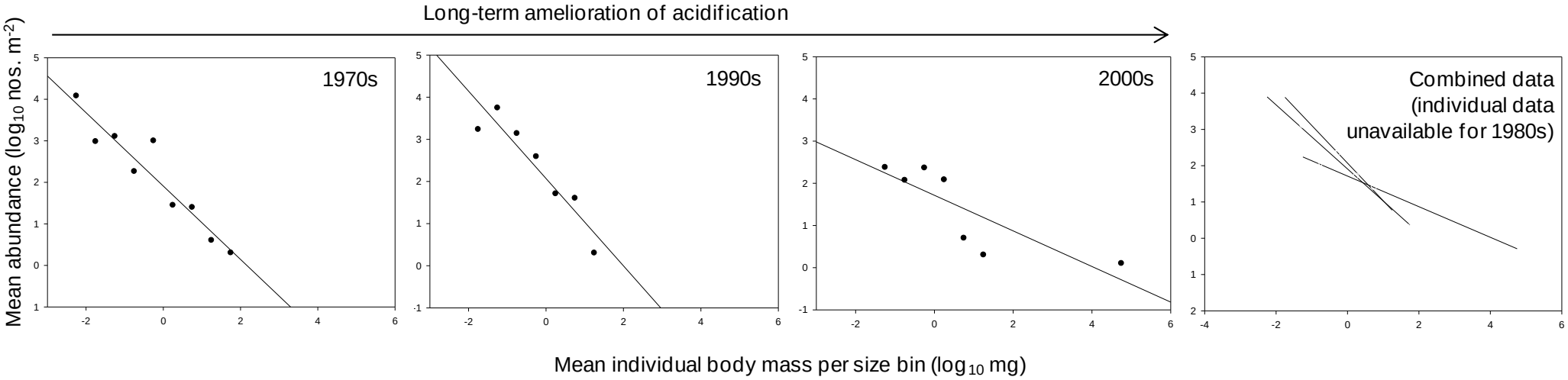

Figure 4.2. Individual Size Distribution (ISD) mass-abundance plots for the Broadstone Stream assemblage (exc. meiofauna) for August 1974, August 1997, August 2006 and all three decades combined. Regression equations as follows: (1970s) y=-0.8x + 2.01, F = 19.7, P < 0.01 (1990s) y=-0.7x + 2.01, F = 16.3, P < 0.01 (2000s) y = -0.4x + 1.7, F = 10.7, P < 0.05. Individual body mass data were unavailable for the 1980s dataset and therefore no ISD plot could be produced (see Methods).

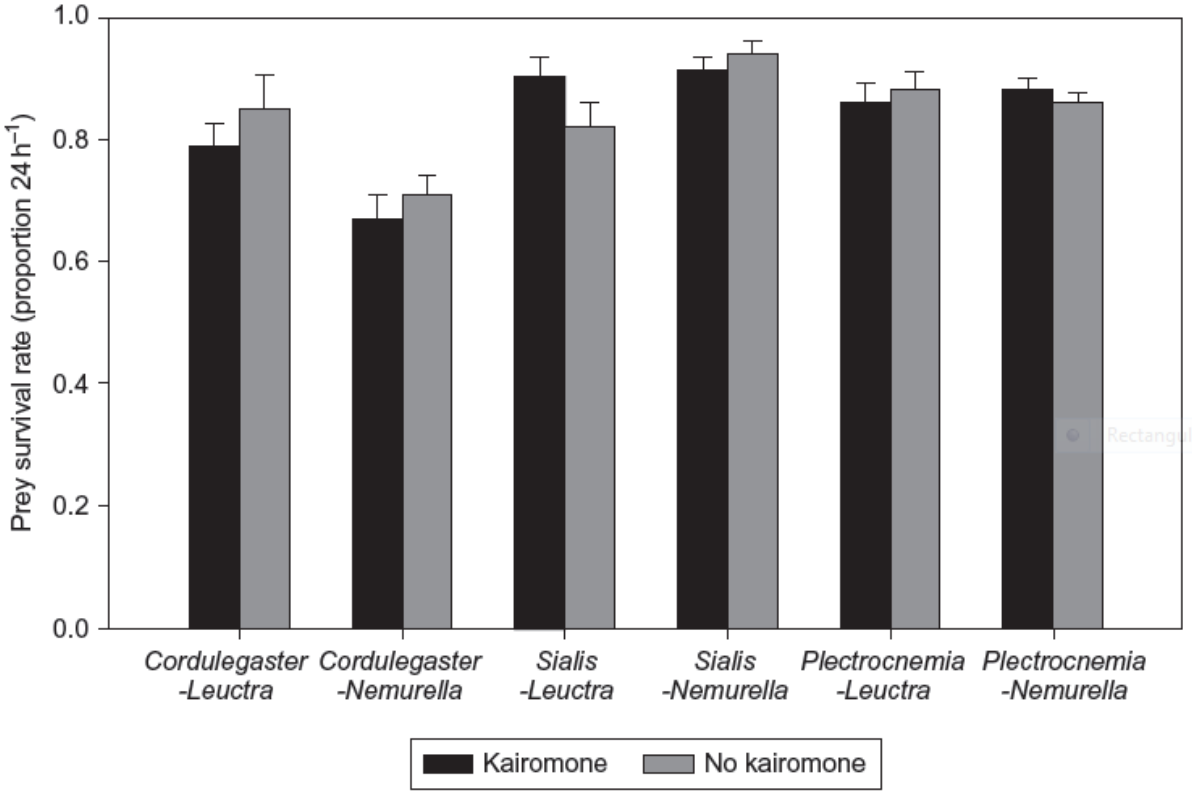

Figure 4.3. Mean survival rates (bars = 1SE) of two common prey species, the detritivorous stoneflies, *N.pictetii* and *L.nigra*, after 24 hours exposure to the three previously dominant large invertebrate predators (*C.boltonii*, *S.fuliginosa* & *P.conspersa*) prior to the trout invasion, in the presence/absence of trout kairomones under controlled laboratory conditions. Statistical analysis was performed on *arcsin*-transformed survival data: although predation rates were higher for *C.boltonii* than for the two smaller species, the presence of trout kairomones had no significant effect on the predator-prey feeding interaction for any of these six common feeding links within the food web.

Discussion

The benefits of consistent and long-term monitoring of a system in response to environmental change are evident in this study, as there have been pronounced shifts in the structure of the Broadstone food web (the most recent of which resulted from the significant and noticeable impacts of trout invasion) that only become apparent in an historical context (i.e after a long time).

Since research on Broadstone Stream began, there has been relatively low species turnover (Woodward *et al.* 2002). As a result, the community has been inferred to be dynamically stable, with noticeable resilience in response to the population irruption of large invertebrate predators (Woodward & Hildrew 2001) and marked robustness compared to other, more circumneutral, streams (Layer *et al.* 2010b). This stability could be supported by a combination of factors, such as abundant detrital basal resources (Dobson & Hildrew 1992), a high degree of generalism in the predator guild (Woodward *et al.* 2005a), a proliferation of weak interactions (Woodward & Hildrew 2001) and apparent competition between prey species (see Chapter Three). However, the sustained amelioration of acidity over four decades in Broadstone has now allowed vertebrate invasion – *S.trutta* generally only establishes populations at pH c 5.4 and above (Jensen & Snekvik 1972) due to toxic effects of hydrogen ions and labile aluminium. This has been observed in some other similar stream systems (Monteith *et al.* 2005; Hildrew 2009). The impact of fish invasions on previously invertebrate-dominated streams can lead to species reductions or extinctions (Schofield *et al.* 1988; Flecker & Townsend 1994; Nakano *et al.* 1999; Townsend & Simon 2006). No species losses in the community have, as yet, been observed in Broadstone, but what is noticeably

clear is that the introduction of *S.trutta* has resulted in a marked decrease in the mass-abundance scaling of the community, both on a species and individual basis, combined with an increase in food chain length and inferred interaction strengths. This is consistent with the first hypothesis, that brown trout invasion will have a significant impact on the structure and function of the Broadstone food web.

This increase in interaction strengths indicates strong top-down pressure on the system, which can destabilise a network (McCann 2000). However, the lack of species extinctions indicates that this effect of direct predation pressure is not evident; at least not in terms of species richness. A reliance on some form of external subsidy could alleviate the effects of strong top-down pressure, by mediating reliance on in-stream production. This link with the surrounding terrestrial system is an important factor in the dynamics of headwater streams (Nakano *et al.* 1999). The presence of a subsidy is evident from the decrease in the mass-abundance slopes at the individual level over time (Fig. 4.2). This is potentially due to a subsidy of terrestrial prey to the trout population (Layer *et al.* 2011); a mechanism that has been shown to support fish in unproductive systems, such as acid streams (Allen 1951; Huryn 1996). In addition, the rise in pH that allowed trout invasion could also have led to increased decomposition of basal resources leading to increased energy availability (Dangles *et al.* 2004; Jenkins *et al.* 2013), which in turn increases 'bottom-up' resources to species higher in the food web. Trivariate food web visualisation shows a progressive lengthening of food chains, from the 1970s to the 2000s, which could well result from an increase in rates of decomposition. I investigate this further in Chapter Five. If this is the case, the effects of top-down and bottom-up processes would be having a simultaneous, consistent effect on the system. Either of these hypotheses (top down or bottom up processes) could potentially

explain why there has not yet been a measurable decrease in the stability of the system, despite evidence for the destabilising effects of trout invasions in general.

In addition, the potential for indirect interactions to mediate any destabilising impact of trout predation could be important (Schmitz *et al.* 1997). However, the lack of any trait-mediated behavioural response observed from experimentally manipulating combinations of predators and prey suggests that the trout are not having an indirect effect on their prey in the stream, consistent with the second hypothesis, that trout impact will be a result of direct, top-down trophic effects as opposed to non-trophic behavioural effects. This correlates with previous studies on Broadstone predators, which showed no significant avoidance responses from acid-tolerant prey species present in the system (Woodward & Hildrew 2002a).

While behavioural avoidance mechanisms have been reported before in response to kairomones (e.g. Abjornsson *et al.* 2001), mainly in more circumneutral systems which are dominated by acid-sensitive species with highly-developed chemosensory apparatus (e.g. mayflies and freshwater shrimps), it could be that kairomones do not elicit such a response in acid-tolerant species, which are adapted to a different chemical environment. Further research would be needed to address this important issue, with Broadstone pH continuing to recover to values approaching those which would support viable populations of these more acid-sensitive species, such as *Baetis rhodani* (see Chapters Two & Three), which do show behavioural response to fish cues (e.g. McIntosh & Peckarsky 1999). It could be that behaviourally-mediated effects will, if acidity continues to ameliorate, eventually have an impact on the influence of brown trout in the system. If so, it would imply that food webs in circumneutral conditions (and above) are not only more complex in terms of readily

observable characteristics, such as numbers of species and feeding links (Woodward 2009; Layer *et al.* 2010b), but also in terms of more subtle indirect interactions, such as behavioural avoidance, which could consequently mediate the interaction strength of observed feeding links. I investigate this further in Chapter Three.

Further work on the consequences of amelioration in the acidity of sensitive fresh waters is needed to expand on the key question of why biological recovery in acidified systems is slower than chemical recovery in formerly acidified sites. However, the data I present here suggest that the internal dynamics of the community could have a significant role. Continued and sustained monitoring of the system is doubtless necessary in order to observe whether the destabilising potential of this voracious generalist predator will have long-term negative consequences for the stability of the Broadstone community, or whether some combination of internal interactions can create an assemblage resistant to significant biotic perturbations, which would in turn potentially explain the ecological inertia apparent in many recovering acid fresh waters.

Chapter Five: Long-term amelioration of acidity accelerates decomposition in headwater streams.

This chapter was published as: **Jenkins GB**, Woodward G, Hildrew AG (2013) Long-term amelioration of acidity accelerates decomposition in headwater streams, *Global Change Biology*, **19**, 1100-1106.

Abstract

The secondary production of culturally acidified streams is low, with a few species of generalist detritivores dominating invertebrate assemblages, while decomposition processes are impaired. In a series of lowland headwater streams in southern England, I measured the rate of cellulolytic decomposition and compared it to values measured three decades ago, when anthropogenic acidification was at its peak. I hypothesised that, if acidity has indeed ameliorated, the rate of decomposition will have accelerated, thus potentially supporting greater secondary production and the longer food chains that have been observed in some systems. I used cellulose test cloth as a standardised bioassay to measure the rate of cellulolytic decomposition, via loss in tensile strength, for 31 streams in the Ashdown Forest over 7 days in summer 2011 and 49 days in winter 2012, and compared this to data from an otherwise identical study conducted in 1978 and 1979. Overall mean pH had increased markedly over the 32 years between the studies (from 5.99 to 6.66). In both the previous and contemporary studies, the relationship between decomposition and pH was strongest in winter, when pH reaches a seasonal minimum. As in the late 1970s, there was no relationship in 2011/12 between pH and decay rate in summer. As postulated, decomposition in winter

was significantly faster in 2011/12 than in 1978/79, with an average increase of 18.1%. Recovery from acidification, due to decreased acidifying emissions and deposition, has led to an increase in the rate of cellulolytic decomposition. This response in a critical ecosystem process offers a potential explanation for one aspect of the limited biological recovery that has been observed so far; an increase in larger-bodied predators including fish and an increase in the length of food chains.

Introduction

The widespread anthropogenic acidification of fresh waters in Europe and North America has had profound ecological effects, including structural changes to communities, such as a loss of sensitive taxa and shifts in the relative abundance of species (Schindler 1988; Hildrew & Ormerod 1995; Kowalik *et al.* 2007; Layer *et al.* 2011; 2012). Many of these structural changes can be ascribed to the direct toxic effects of acidity and its associated chemical conditions (e.g. Hildrew & Ormerod 1995). Further, several ecosystem processes, such as decomposition (Chamier 1987; Hildrew *et al.* 1984; Dangles *et al.* 2004; Simon *et al*. 2009; Pye *et al.* 2012), primary production (Mulholland *et al*. 1986; Niyogi *et al*. 2002) and bacterial production (Mulholland *et al*. 1992), are sensitive to mineral acidity and their rates seem generally reduced in acidic waters, possibly with ramifying indirect effects through freshwater food webs. Thus, the information available on the benthic secondary production of acidified streams also suggests that this is very low (Krueger & Walters 1983; Griffith *et al.* 1994; Pretty *et al.* 2005; Stead *et al.* 2005; Woodward *et al.* 2005a), probably partly as a result of the poor food quality of detritus in acidified streams (e.g. Groom & Hildrew 1989).

The ecological effects of acidification, therefore, can be ascribed to a mix of direct (toxicological) and indirect (via trophic) interactions. In this context, the effects of a chemical reversal of acidification are of particular interest, since the alleviation of toxic effects is likely to lead to a direct ecological reversal, whereas prominent indirect effects are likely to cause lags, hysteresis and non-linearities in recovery (Ledger & Hildrew 2005; Layer *et al*. 2010a, b; 2011, 2013). Following the implementation of international agreements, there have been marked reductions (38 -72% from the peak in the late 1970s and early 1980s) in sulphur emissions in Europe and North America, and these have led in turn to declines in acid deposition (wet sulphate) by 10 - 45% (Lynch *et al.* 2000; Fowler *et al.* 2005). Consequent chemical recovery in surface waters is becoming increasingly evident in most regions with acidified freshwater bodies (Skjelkvåle *et al.* 2005; UKAWMN 2010).

The biological responses to chemical recovery have so far proved patchy and somewhat equivocal (Yan *et al*. 2003; Monteith *et al.*, 2005; Ormerod & Durance 2009; Johnson & Angeler 2010; Murphy *et al.* 2013) and have not included a simple return of the expected acid sensitive invertebrates to streams, for instance. Of the several signs of biological recovery that have been apparent, Monteith *et al*. (2005) reported an increase in the numbers of several larger-bodied invertebrate predators in the sites of the UK's Acid Waters Monitoring Network, and a recovery of fish populations in a few. In the particularly well characterized acidic Broadstone Stream in southern England, the community has been dominated, since the 1970s, by a series of progressively larger bodied predatory invertebrates followed by the recent invasion by brown trout (*Salmo trutta*) of this formerly fishless stream (Woodward & Hildrew 2001; Woodward *et al.* 2005a; Hildrew, 2009; Layer *et al*., 2011). These invasions and irruptions by large-bodied species inevitably led to a lengthening of food chains (Woodward & Hildrew 2005a; Layer *et al.* 2011) and presumably in secondary production.

Hitherto, there have been few assessments of ecosystem processes in acidified fresh waters consequent upon their chemical recovery (Simon *et al*. 2009). With regard to decomposition processes, McKie *et al.* (2006) and Merrix *et al.* (2006) both found that microbial decomposition increased upon liming, although I know of no attempts to assess whether decomposition rate has responded to the chemical amelioration of acidity as a result of direct emission reductions. Historic data on decomposition processes in streams are rare, but Hildrew *et al.* (1984) measured the rate of cellulolytic decomposition in a group of 32 stream sites, across a range of acidities, in southern England (the Ashdown Forest) in 1978/79, when anthropogenic acidification was at its peak. Using the rate of loss of tensile strength of cellulose test cloth as a measure of decomposition they found a significant positive relationship in winter with pH. Like many others, this area of southern Britain has seen a marked reduction in acidifying deposition since that time (UKAWMN 2010). One of the 32 sites, Old Lodge, is part of the UK Acid Waters Monitoring Network and its chemistry and biota have been studied since 1988 when mean annual pH was less than 4.5. Over the following 21 years it has increased by 0.7 pH units to over 5.1 (in 2009).

There is, therefore, a very valuable background assessment of cellulolytic decomposition at >30 sites more than 30 years ago, at peak acidification, in an acid-sensitive area and in which there have now been marked reductions in deposition and chemical recovery. This gave an opportunity to make contemporary assessments of decomposition at these sites, using identical methods. It can be hypothesised that, if acidity has indeed ameliorated, the rate of decomposition will have accelerated, thus potentially supporting greater secondary production, and the conspicuous lengthening of food chains that has been observed in some freshwater systems recovering from acidification (e.g. Layer *et al.* 2011).

Methods

Study site

The Ashdown Forest of southern England ($51^{o}05'$N, $0^{o}03'$E) contains the spring-fed headwaters of two rivers, the Sussex Ouse (flows south into the English Channel) and the Medway (flows north to joins the Thames estuary). The watershed between the two river systems lies in the centre of the Weald in SE England, on low hills of soft, fine sandstone (Ashdown Sands) that provides little acid-neutralising capacity (Hildrew 2009). Thirty-four sites on streams (first to third order) within the Ashdown Forest, offering a range of acidity from profoundly acidic to circumneutral (the differences largely explained by land use), were used by Hildrew *et al.* (1984) for their original measurements, Access to three of those original sites is now prohibited, but the remaining 31 were again included here to measure cellulolytic decomposition (see Table 5.1 for background).

At two of these 31 remaining sites, one acidic and one circumneutral, I carried out more detailed measurements of decomposition, over a time course, and compared two sources of cellulose (see below for details). These were sites 6 (Old Lodge) and 10 (Crowborough Warren) of Townsend *et al*. (1983), which have a contemporary mean pH of 5.63 and 7.17, respectively.

Environmental data

The physicochemical characteristics of each stream (water temperature, oxygen, calcium, nitrate and iron concentrations) were measured in 1977 by Townsend *et al.* (1983). pH was measured in the field using a portable battery-operated pH meter (Beckman Coulter Inc., Brea, California). In 2011/2012, four spot pH and temperature values were taken in the field in July 2011, October 2011, January 2012 & April 2012 using a portable battery-operated pH pocket meter (WTW, Weilheim, Germany), with a two-point calibration (technical buffers at pH 4.01 and 7), to obtain an overall mean for the exposure period, after the timings of Townsend *et al.* (1983). In addition, a maximum-minimum thermometer was placed in each stream and the median temperature during each experiment was estimated at the end. Unfortunately, water temperature data were not available for the winter testing period in the study of Hildrew *et al.* (1984). To compensate for this, historical air temperature data were obtained from the Meteorological Office (Exeter, UK) UKCP09 5km grid square historical dataset. This dataset was available as mean monthly air temperature across the Medway & Ouse catchments. I then calibrated between these mean air temperatures to corresponding mean water temperatures, by using contemporary mean air and water temperatures from Sites 6 and 10, taken during the more detailed measurements of decomposition, in order generate a linear regression equation (see Fig. 5.1). This was then converted using Meteorological Office monthly air temperature data to historical mean water temperature using this regression, and these estimates were then used for temperature adjustments to the winter 1979 data.

Cellulolytic decomposition

As a measure of cellulolytic decomposition, strips cut from Shirley cellulose test cloth were used (Shirley Institute, Manchester, UK). The adoption of cellulose test strips as a standard for estimating decomposition was pioneered in soils (Kuzniar 1948, Latter & Howson 1977), for which it remained in use for several decades (Latter & Walton 1988, Treonis *et al.* 2002). They were also adapted for use in aquatic systems (Egglishaw 1972, Hildrew *et al*. 1984.), and cellulose strips remain popular in aquatic ecology as a bioassay (Boulton & Quinn 2000, Tiegs *et al.* 2007, Imberger *et al.* 2010). Shirley test cloth is almost pure cellulose (99.63%, with the remainder being trace amounts of wax and fat, Hildrew *et al.* 1984) – it is not a direct mimic of the much more heterogeneous allochthonous detritus, which are predominantly leaf fragments in these systems, but is readily standardised and replicated (Tiegs *et al.* 2007). Shirley test cloth is no longer commercially available (Imberger *et al.* 2010), but we obtained sufficient material to repeat the original 1970s study (Hildrew *et al*. 1984). Decomposition was assessed in that earlier study as the loss in tensile strength, and this measure was therefore also adopted here (see below). Control strips of the Shirley test cloth broke at 336.86N (± 4.7 SE), which compares well with the controls of Hildrew *et al.* (1984) (338N ± 1.6 SE), indicating no degradation in the test cloth structure over time.

As in the original study, two trials were conducted, to estimate decomposition in both 'Summer' and 'Winter'. On each occasion, three Shirley cloth strips, each measuring 20 mm x 80 mm, were placed in a shallow riffle section of each stream, attached by garden wire to metal re-bars driven into the substratum, which left the strips positioned in the water column above the substrate. In July 2011, the strips remained *in situ* for seven days, and in January 2012 for 49 days, matching exactly the sampling periods and duration in Hildrew *et al.*

(1984); they demonstrated from preliminary experiments that shorter exposure would have resulted in negligible decomposition and longer exposure in complete fragmentation/loss of the strips.

In a subsidiary study, I aimed to test whether loss of tensile strength followed a linear or exponential decline. The latter is to be expected based on the decay model widely used in previous work on decomposition processes (e.g. Petersen & Cummins 1974, Hladyz *et al.* 2009), but it was important that this was determined for statistical analysis and for any potential transformation of the data. Further, Imberger *et al.* (2010) suggested that unbleached calico could be used as an effective cellulose substitute for the now obsolete Shirley test cloth, and the two were compared in their decay and to further calibrate calico under our field conditions. It would also allow more direct comparison with other studies. Therefore, seven sets of test cloth, each comprised of three Shirley cloth strips and three calico strips of similar dimensions, were placed into an acid stream (Old Lodge, site 6 of Townsend *et al.* 1983 and site 13 in the UK's Acid Waters Monitoring Network) and a circumneutral stream (Crowborough Warren, Site 10 of Townsend *et al.* 1983) (see Table 1). One set was retrieved from each site after 1, 2, 3, 6, 12, 24 and 48 days of exposure.

At the end of all sampling periods, strips were recovered from the streams, rinsed thoroughly in tap water and oven-dried to constant mass at 65°C, the loss in tensile strength then being tested after Hildrew *et al.* (1984). Tensile strength was measured using a Hounsfield machine (Maywood Instruments Ltd., Basingstoke, UK) with a 25kN load cell and 40mm test gap. One estimate of breaking strain was obtained per strip. Three untreated strips (which were also rinsed thoroughly in tap water after being taken to the field and returned, then oven-dried to constant mass before tensile strength testing) were used as controls for any loss of strength from transport and washing in tap water.

Statistical analyses

As this study was focussed on the effect of pH, all percentage loss in tensile strength data were corrected for differences in water temperature by dividing by cumulative degree days, to avoid confounding the results by any differences between the studies. The following formula was applied:

-ln (Proportion remaining tensile strength (N)) / Cumulative degree-days (^{o}C).

Degree days were calculated using the mean water temperature from maximum-minimum thermometers attached to the metal re-bars driven into the substratum.

Comparisons of physicochemical factors between years were made using one-way ANOVA. Comparisons between pH and temperature-corrected loss in tensile strength and different cellulose cloth types were made on winter data only using one-way ANCOVA, with pH as covariate and year as the fixed main effect, with no interaction terms fitted. Linear regressions were used on log-transformed time series data and Shirley/calico comparisons and tested for statistical significance using SigmaPlot Version 12 (SyStat Software Inc, Chicago, U.S.A). All other statistical analyses were performed using MINITAB Version 14 (Minitab Inc., Pennsylvania, U.S.A)

Results

There has been a significant increase in average pH (by a mean of 0.7 pH units ± 0.08 SE) across the 31 sites, from 6 in 1978/79 to 6.7 in 2011/2012 ($F_{1, 56} = 21.93$, $P < 0.001$) (Fig. 5.2). Summer water temperature in 1978 (mean = 16.67 °C ± 0.29 SE) was higher than in 2011 (mean = 14.34 °C ± 0.17 SE) ($F_{1, 56} = 32.91$, $P = < 0.001$), but there was no significant difference between the years in winter water temperature. At the two sites at which the time course of decomposition was assessed, decay fitted a negative exponential model, a constant fraction of tensile strength being lost per unit time (Fig. 5.3). Accordingly, all tensile strength loss data were log-transformed before further analysis.

In summer, there was no relationship between pH and decay rate. Contemporary raw loss in tensile strength ranged from 0.97% to 43.59% relative to the unexposed control, with a mean loss of 13.5% (± 2.55 SE), which was lower than in the corresponding 1978 study, which had a mean strength loss of 23.07% (± 3.88 SE) ($F_{1,56} = 6.83$, $P < 0.05$)(Fig. 5.4a). When the raw summer data were corrected for temperature (higher in 1978 than in 2011), there was no overall difference in decay rate between years, and no significant relationship in either study between pH and temperature (Fig. 5.4a). Thus, decay rate, corrected for temperature, was similar between years and clearly neither the rate nor the range of rates were driven by summer pH, which had risen considerably.

In winter, decay rate clearly increased with pH in both years and, since pH was higher in 2012 than in 1979, decay was more rapid in the recent experiment (Fig. 5.4b). In winter, the contemporary loss in tensile strength ranged from 16.84% to 100% (the latter equivalent to c 45-50% mass loss, Tiegs *et al*., 2007) relative to the control, with a mean loss of 73.9% (± 4.46 SE). This was greater than in the 1979 study, where loss in tensile strength ranged from 3% to 100% with a mean loss of 46.9% (±5.88 SE) ($F_{1,60} = 4.19$, $P = 0.045$). In the winter

data, corrected for minor differences in temperature between years, decay rate increased with pH in both years, though there was still a significant difference in the relationship between years (Table 5.2; Fig. 5.4b), with decomposition apparently accelerating with pH at a somewhat greater rate in the historical data.

A comparison of the tensile strength of the two test materials showed that calico was consistently less strong than Shirley test cloth (see Fig. 5.5). However, there was a clear linear relationship between the two and the data for periods of exposure from 1 to 40 days in both an acidic (pH 5.6) and a circumneutral stream (pH 7.2) all fitted the same regression, with decomposition much faster in the latter.

Tables & Figures

Table 5.1. Values of physicochemical variables in the 34 stream sites, from Townsend *et al.* (1983). An asterisk indicates stream is part of the Sussex Ouse; remaining are part of the Medway. Sites 16, 30 and 31 are currently on Ministry of Defence land and were excluded from this study.

				Discharge ($m^3 s^{-1}$)		Temperature (°C)					Iron ($mg l^{-1}$)	
Site	**Distance (km) from headwater**	**Mean annual pH**	**Range of pH**	**Max**	**Min**	**July**	**Annual Range**	**Oxygen ($mg l^{-1}$)**	**Calcium ($mg l^{-1}$)**	**Nitrate ($mg l^{-1}$)**	**Soluble**	**Total**
1	0.61	5.6	4.5-6.0	0.03	0.007	14.4	7.7	5.1	8.1	2.3	1.0	6.8
2	1.75	6.3	5.6-6.8	0.05	0.006	16.4	9.9	7.2	17.8	4.4	0.3	0.3
3	1.77	5.2	4.3-6.1	0.06	0.002	14.2	7.9	7.6	10.9	6.5	0.7	1.1
4	1.82	5.0	4.1-5.7	0.06	0.036	15	8.6	7.6	11.8	2.3	1.4	1.8
5	3.90	5.0	4.4-6.8	0.10	0.012	17.8	11.2	7.0	12.4	12.9	0.2	0.6
6	1.72	4.8	3.6-5.6	0.04	0.012	15.6	9.4	7.8	6.9	2.4	0.3	0.4
7	2.40	5.8	4.2-6.8	0.10	0.021	16.3	10.1	9.0	9.9	2.4	1.4	3.0

8	2.10	5.8	4.6-6.5	0.08	0.003	15	8.6	8.9	5.0	0.8	0.8	2.0
9	1.82	5.2	3.8-5.7	0.05	0.001	15.8	9.3	8.4	4.6	0.7	0.1	0.2
10	1.27	6.1	5.4-6.5	0.04	0.004	16.2	10.2	2.9	48.5	72.9	0.1	0.4
11*	0.56	6.3	5.6-6.6	0.03	0.002	15	7.9	7.6	17.1	7.3	0.2	0.6
12*	1.21	6.2	5.1-6.8	0.12	0.006	14.3	7.8	7.0	16.2	6.3	1.5	2.6
13*	1.64	5.5	4.1-6.4	0.07	0.011	17	10.8	9.0	5.5	1.4	0.2	0.6
14*	1.80	6.2	5.3-6.4	0.10	0.028	18.4	12.1	8.2	10.2	3.6	0.1	0.6
15*	2.58	6.2	5.5-6.9	0.20	0.029	16.1	9.3	6.9	11.8	3.4	1.0	1.7
16	7.46	5.9	4.7-6.4	0.26	0.022	19	12.7	6.8	11.6	4.2	0.2	0.9
17	3.67	6.4	5.2-7.3	0.15	0.006	14.7	8.3	7.9	14.2	5.1	0.3	1.5
18	4.73	5.9	5.0-6.3	0.25	0.009	16.3	10	7.8	12.2	5.8	0.2	0.8
19	4.83	5.9	4.6-6.5	0.27	0.032	16.1	9.7	7.0	10.9	5.2	2.8	7.3
20	5.41	5.8	5.0-6.3	0.21	0.029	16.7	10.8	7.9	11.7	4.6	1.2	1.7
21*	3.19	6.5	5.5-7.0	0.19	0.054	17.3	11	8.8	12.8	4.6	0.5	1.3

22*	4.88	6.3	5.3-7.3	0.26	0.031	16.9	10.8	7.6	17.6	6.7	0.4	0.7
23*	6.05	6.4	5.3-7.4	0.42	0.043	18.7	12.3	8.1	19.8	8.0	0.2	0.9
24*	3.14	6.3	5.4-7.1	0.24	0.010	15.5	8.9	8.1	14.1	3.2	0.8	1.0
25*	5.29	6.9	6.4-7.8	0.30	0.017	16.2	9.6	8.8	20.1	9.5	0.2	1.4
26	9.54	6.1	4.4-6.8	0.39	0.033	17	10.4	8.0	10.9	1.9	0.3	1.0
27	11.08	6.3	5.1-7.0	0.35	0.043	16.6	9.8	6.2	11.9	1.1	0.5	1.3
28	6.30	6.5	5.6-7.4	0.41	0.209	15	9	4.1	16.1	15.6	0.4	1.2
29	9.29	6.3	5.4-6.7	0.47	0.091	16.8	10.2	7.3	15.9	12.1	0.2	1.0
30	3.01	6.0	4.8-6.5	0.08	0.008	21	14.4	7.0	10.9	4.8	0.3	1.0
31	6.55	5.9	4.6-6.7	0.32	0.023	21.1	14.1	8.1	13.2	4.7	0.2	0.8
32	2.13	5.6	4.5-6.0	0.09	0.002	18.3	12.4	7.9	9.6	3.8	0.2	0.5
33	8.22	6.4	5.4-7.0	0.41	0.068	16.6	9.9	7.5	16.8	14.7	0.2	1.5
34*	2.35	6.4	5.3-7.1	0.12	0.022	19.5	13.2	7.4	13.8	5.1	0.4	2.0

Table 5.2. Results of ANCOVA on temperature-adjusted winter decomposition rates with year as fixed effect and pH as covariate.

		d.f	**F-ratio**	**P**
Winter	pH	1	19.56	< 0.001
	Year	1	4.93	0.029
	Error	56		
	Total	58		

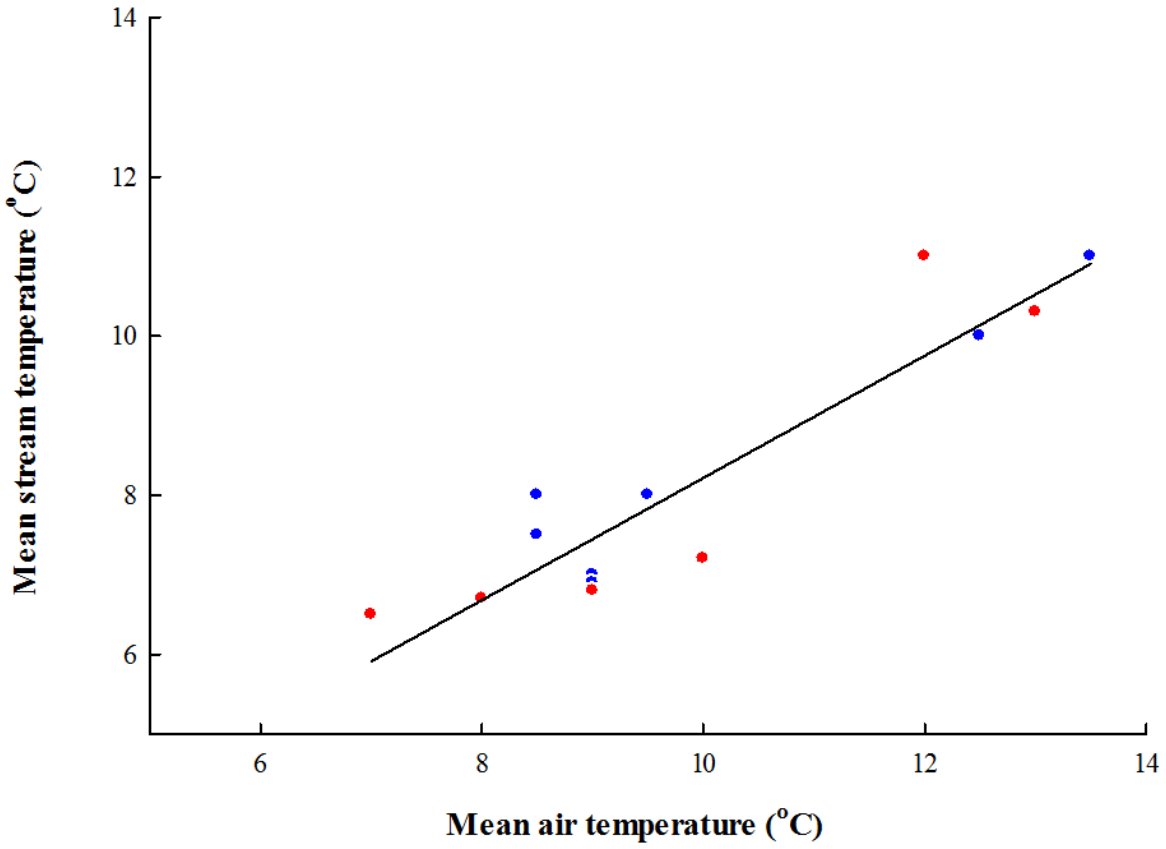

Figure 5.1. Calibration between mean air and stream temperatures in degrees Celsius from (a) an acid stream (red) and (b) a circumneutral stream (blue). Fitted line from linear regression ($F_{1,13} = 71.8$, $r^2 = 0.86$, $P < 0.001$) with equation $y = 0.799x + 0.542$.

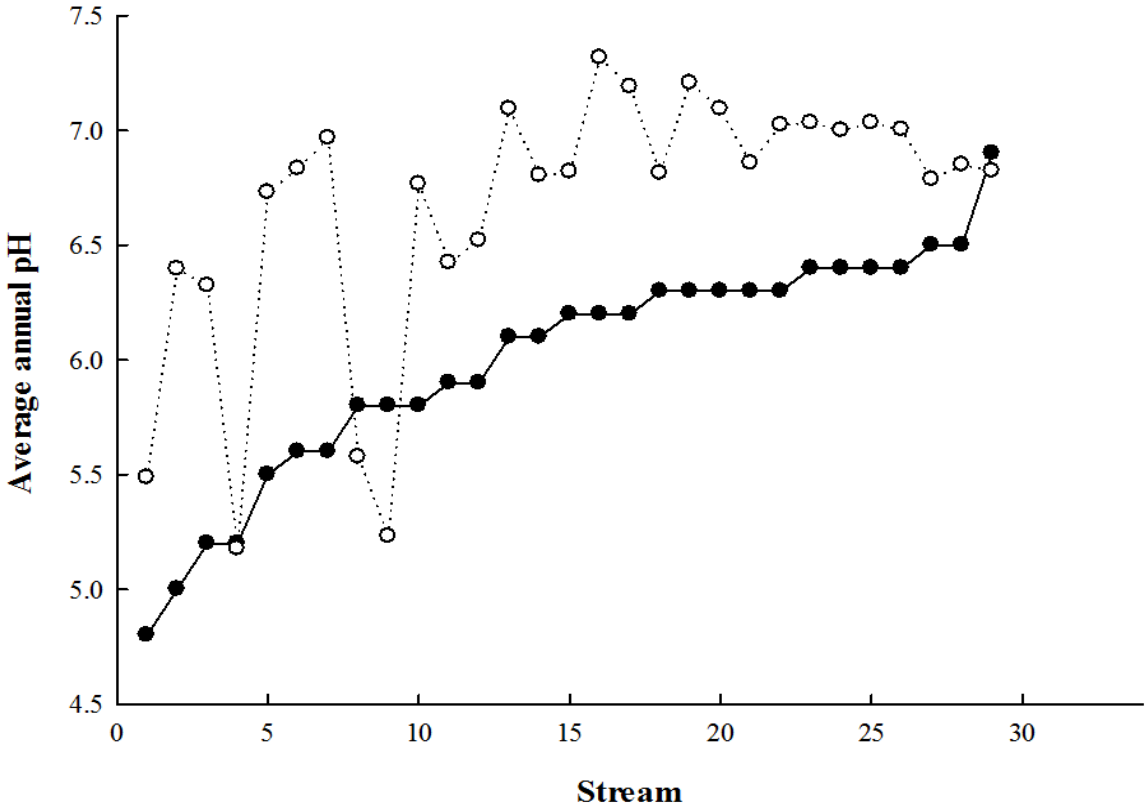

Figure 5.2. Average annual pH of Ashdown Forest survey stream sites for 1978/9 (black circles) and 2011/12 (white circles). Streams are ranked according to initial mean annual pH in 1978/9.

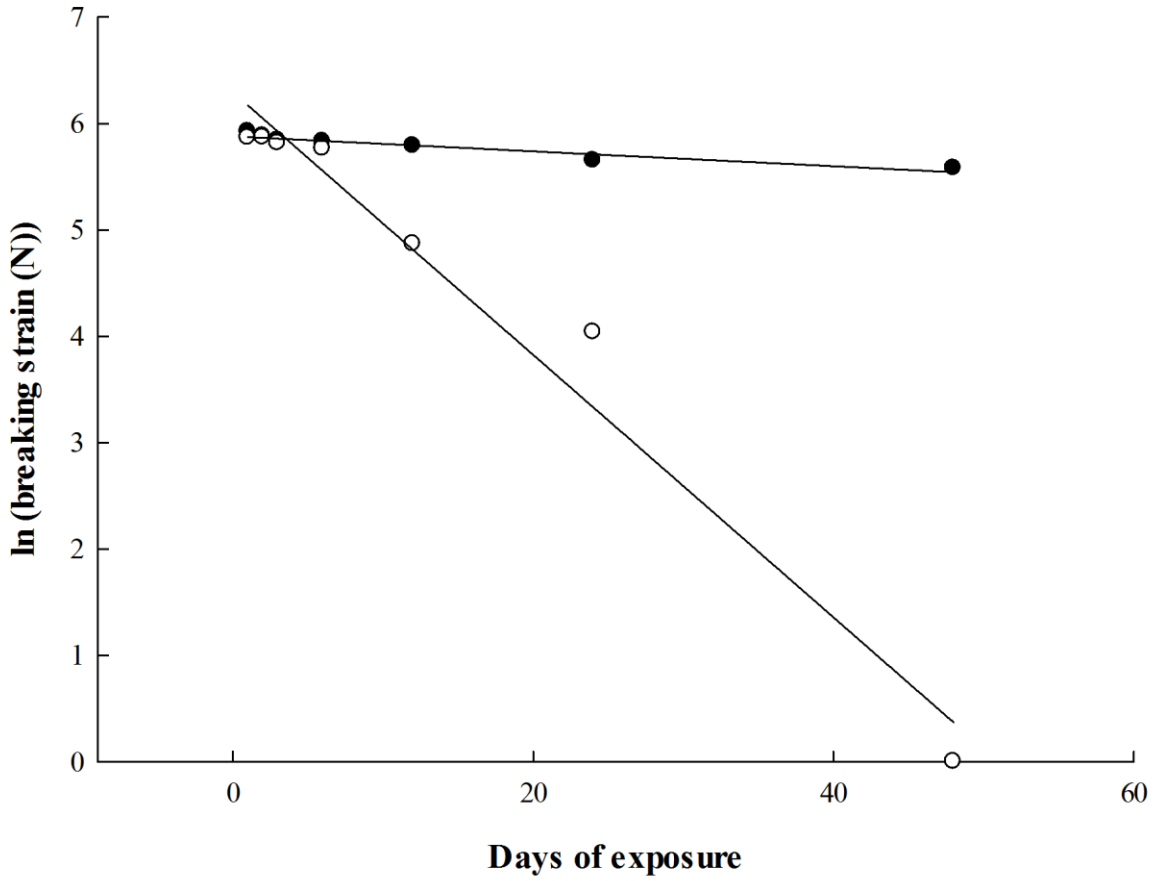

Figure 5.3. Rate of decline in tensile strength for Shirley cellulose test cloth after 1, 2, 3, 6, 12, 24 and 48 days of exposure in (a) an acid stream (black circles) and (b) a circumneutral stream (white circles). Fitted lines are from linear regression after log-transformation: a - ($F_{1, 6} = 163.23$, $r^2 = 0.97$, $P < 0.001$), b - ($F_{1, 6} = 53.67$, $r^2 = 0.91$, $P < 0.001$).

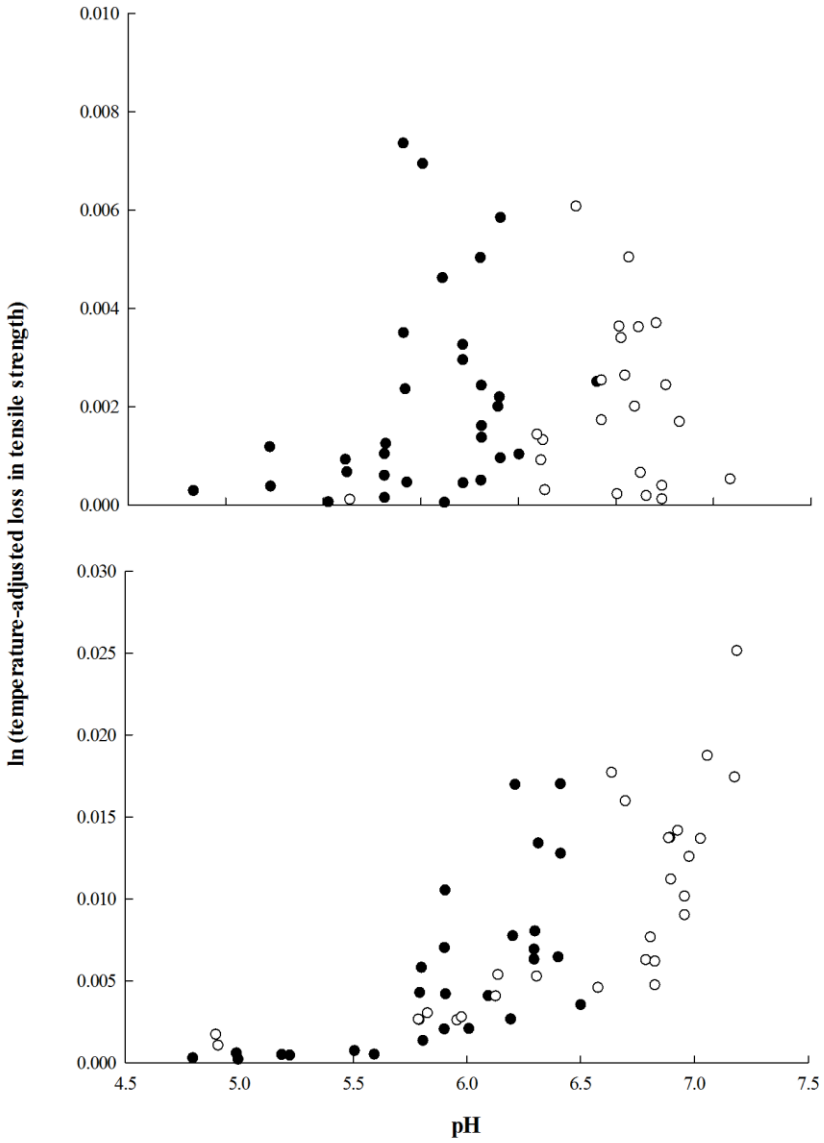

Figure 5.4. Temperature-adjusted log-transformed tensile strength loss of Shirley cellulose test cloth against stream pH for: (a) 7 days exposure in summer 1978 (black circles) and summer 2011 (white circles) and (b) 49 days exposure in winter 1979 (black circles) and winter 2012 (white circles). Note difference in length of y-axes.

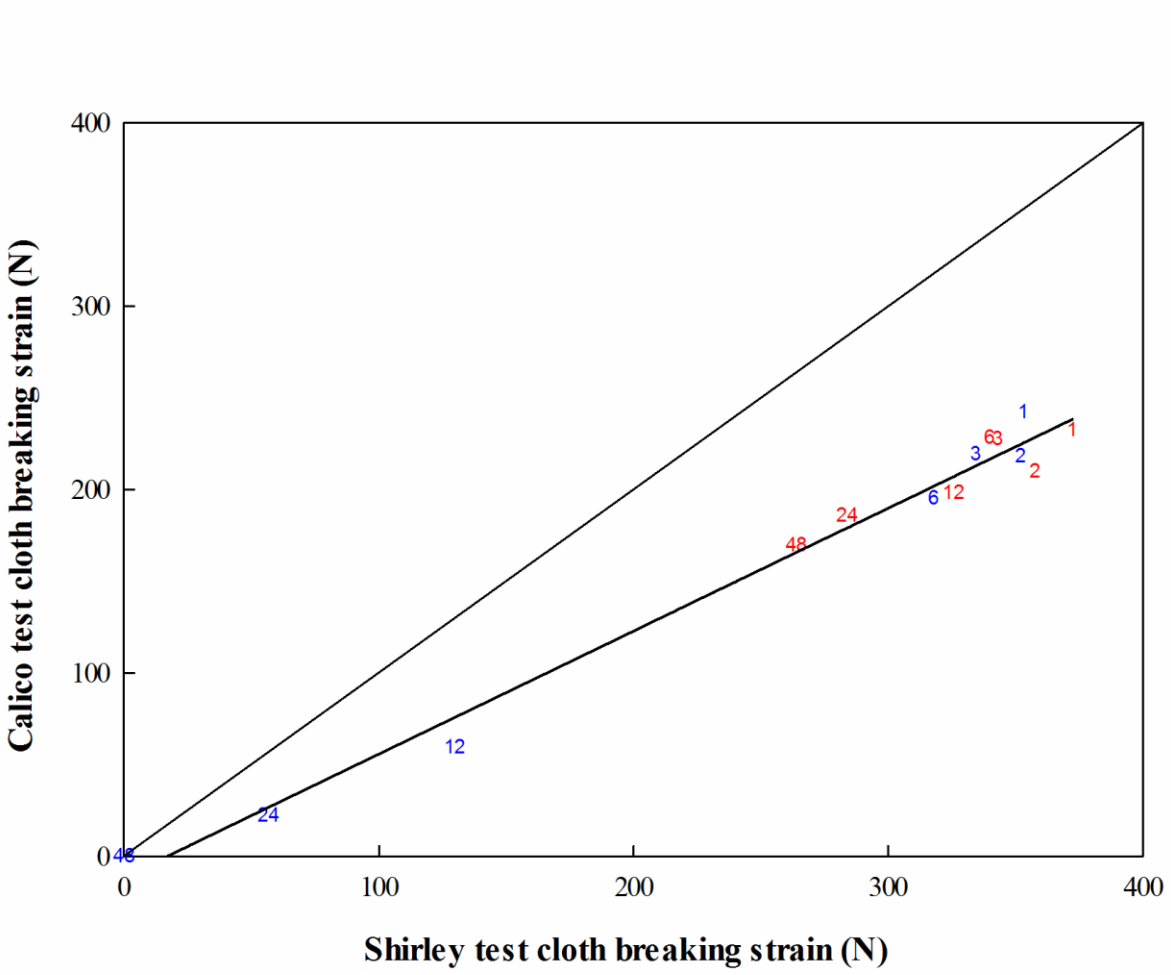

Figure 5.5. Comparison between Shirley and calico test cloth tensile strength loss after 1, 2, 3, 6, 12, 24 and 48 days of exposure in (a) an acid stream (red) and (b) a circumneutral stream (blue). Fitted line from linear regression (one-to-one line shown for purposes of comparison).

Discussion

It is now well established that litter decomposition is inhibited in acidified streams (e.g. Burton *et al.* 1985; Chamier 1987; Groom and Hildrew 1989; Mulholland *et al.* 1992; Griffith and Perry 1993; Dangles & Guérold 2001; Dangles *et al.* 2004; Baudoin *et al*. 2008). In an earlier study of cellulolytic decomposition using Shirley test cloth (Hildrew *et al.* 1984), pH was a strongly significant driver of decomposition in winter, and this held true 32 years later. The much higher average pH in 2010/2011 compared with 1977/1978, however, was associated with a greater overall rate of loss in tensile strength in the contemporary study and this indicates a widespread increase in decomposition in formerly acidified streams. This is the first demonstration of an acceleration in this key ecosystem process consequent upon a widespread reduction in sulphur deposition and chemical amelioration of streams (Simon *et al.* 2009). This increase in decomposition provides a likely explanation for increases in food chain length, presumably supported by greater secondary production, that have been observed recently in the food webs of some recovering streams (Monteith *et al.* 2005; Woodward *et al.* 2005a; Layer *et al.* 2011).

The test cloth used is pure cellulose, containing none of the secondary compounds, proteins, lipids or intrinsic mineral nutrients that are variably found in natural leaf litter (Tiegs *et al.* 2007). Due to their mode of exposure here, in the flow, above the substratum and attached to metal re-bars, they are not easily colonised by invertebrates. Indeed, van Gestel *et al.* (2003) showed no relationship between the loss of strength of cellulose cloth in soils and the density of invertebrates, so it can be assumed that their decay is due overwhelmingly to cellulolytic microorganisms (Tiegs *et al.* 2007). Although alternative forms of cellulose now exist, in the form of tablets, which have advantages over test cloth in that their chemical composition can

be manipulated, and are readily consumed by sediment-dwelling animals (Kampfraath *et al*. 2012), for purposes of consistency it was decided to replicate the methods of the previous survey.

Two further aspects of the data deserve comment. First, why is decomposition dependent on stream pH in winter but not in summer? One possible explanation is that the pH of the most acidified streams in the Ashdown Forest is at its seasonal highest in summer (Hildrew 2009), possibly as a result of de-acidifying reactions in the streams, such as iron reduction which consumes hydrogen ions (Hildrew & Ormerod 1995), which in turn reduce the toxicological effects of low pH on stream biota. This effect is not very clear in the data-set, however, although the number of sites overall with a pH below the biological critical value of c. 5.5 was slightly less in summer (Fig. 5.4). Nevertheless, this is not a complete explanation. Alternatively, cellulolytic decomposers may be more active at higher temperature, and less affected by acidity, nutrient shortage or substrate quality (e.g. Ferreira & Chauvet 2011; Fernandes *et al.* 2012), and the results do suggest a reduction in any inhibition of decomposition by acidity in the summer.

A second outcome is that, set against an overall increase in pH and decomposition in the contemporary data, the rate of decay in the 1977/78 data was apparently actually faster at a common intermediate pH than in the recent experiment (the relationship between pH and decay differed between years; Table 5.2). Since temperature differences have been corrected for, this is not an explanation. A possible factor is nutrient supply. Monitoring of N concentration in precipitation and bulk deposition in the area (Barcombe Mills, co-located with the Ashdown Forest: UKAWMN, 2010) from 1988 to 2008 showed that total N concentration fell from about 1.4 mg N L^{-1} to <0.5 mg N L^{-1} and bulk deposition from >0.002 to <0.001 mg N m^{-2} d^{-1} (both significant declines). In sites not affected by local inputs of N

(most have wooded or moorland catchments) this suggests a reduction in N supply between 1978/79 and 2001/12, and N-deposition certainly was no lower in the late 1970s than the late 1980s, when monitoring of deposition began. Environmental nitrogen is an important factor in determining the rate of decay in streams (e.g. Webster & Benfield 1986; Suberkropp & Chauvet 1995), while Hobbie *et al.* (2012) recently showed that cellulolytic decomposition is accelerated by external N in forest soils (though it inhibited decomposition of more recalcitrant fractions of the litter).

Overall, this study provides evidence that increased pH in streams recovering from acidification has led to an increase in cellulolytic decomposition, a fundamental ecosystem process. This could result in increased secondary production, food chain length and trophic height and potentially explain some aspects of the ecological recovery observed to date. While factors such as morphological constraints, ecosystem size and dynamical stability can also have an effect on food chain length (Post 2002), energy availability (as affected by the rate of cellulolytic decomposition) seems likely to be an influential constraint in this system. This study also demonstrates the advantages of hypothesis-driven, sustained research and monitoring (Hildrew 2009), which enabled observation of a process of changes across a substantial number of sites and years, while also providing the first evidence of long-term recovery in a key ecosystem process after three decades of reductions in industrial acidifying emissions. If chemical recovery of acidified streams continues, including a decline in acid episodes (Kowalik *et al*. 2007; Pye *et al*. 2012), it can be expected that more signs of biological recovery will emerge as changes in such ecosystem processes begin to translate into the recovery of assemblages higher in the food web.

Chapter Six: General Discussion

The overall aim of this thesis was to examine the inertia in biological recovery of acidified fresh waters from the acidifying events of the 19th and 20th centuries. The widespread amelioration of acidity in freshwater systems, in a purely chemical context, is now well documented (e.g. Fowler *et al.* 2005; Kowalik & Ormerod 2006; UKAWMN 2010; RoTAP 2012; Murphy *et al.* 2013), whereas compelling and widespread evidence for any corresponding biological response is lacking (Battarbee *et al.* 2013). Various theories have been suggested as to why that may be – the main three being recurring acid episodes, limitations on coloniser dispersal and intrinsic food web resistance (see Chapter One for details) - but there is no clear consensus as to which is responsible. In this thesis, I have aimed to address this gap in our knowledge by examining the effects of acid amelioration at different levels of organisation within freshwater systems, specifically headwater streams, ranging in scale from individual interactions to whole ecosystem-level responses. To do this, I used a model system (Broadstone Stream, SE England). It has been regarded as dynamically stable due to low species turnover (Woodward & Hildrew 2001), which has been observed over several decades of extensive study (Hildrew 2009). Broadstone also has one of the best-described food webs in the world (Woodward *et al.* 2005a), which makes it an ideal study site for examining responses to amelioration of acidity, at a variety of organisational levels.

A key aspect of research into study systems such as Broadstone in recent years has been a shift from species presence/absence studies towards increasing emphasis on the interactions between these species (Woodward 2009), and the role that such interactions play is a key focus of this thesis. To do this, I used a combination of laboratory and field experiments, combined with historic survey data, and each of the data chapters covered an increasingly

higher level of organisation within a freshwater network. In Chapter Two, I demonstrated a population-level resistance to new taxa colonising acidified systems, driven by antagonistic direct interactions between predators and prey, which is manifested by universal Type III density-dependent functional responses across the resident macro-invertebrate predator guild. Trophic interactions such as these have been demonstrated play a key role in ecosystem dynamics (Martinez 1991; Pawar *et al.* 2012). In Chapter Three, I presented further evidence of this role of interactions in food web stability, by using a basic network-level approach to examine more subtle indirect interactions, which are increasingly realised to be important in regulating trophic dynamics (Beckerman *et al.* 1997; Petchey *et al.* 2008; Ings *et al.* 2009; Kefi *et al.* 2012). I demonstrated that apparent competition between resident acid-tolerant species and acid-sensitive potential colonisers could prevent high densities of novel species from establishing. In Chapter Four I took a different approach, by switching from experimental testing of interactions to observing the effects of shifts in the magnitude of such interactions in a natural environment. Broadstone has recently been invaded by a large acid-sensitive vertebrate, brown trout (Layer *et al.* 2011), which is a voracious predator and has the potential to cause strong top-down destabilising effects on a system (Nakano *et al.* 1999; Townsend & Simon 2006). This would appear to be the case in Broadstone, as long-term data analysis indicates the invasion of trout has led to shifts in mass-abundance scaling at both the species-averaged and individual size distribution, combined with increase in mean interaction strength. This increase is suggestive of top-down control of the community assemblage in response to increasing pH enabling colonisation by novel predators. However, there is also evidence of bottom-up responses to ameliorating acidity, as I demonstrated in Chapter Five that rates of microbial decomposition, a key ecosystem-level process, are increasing as pH rises throughout the catchment. This suggests that energy limitation, through impaired microbial conditioning of detrital input, is not a factor in constraining ecological recovery,

and it is therefore likely that energetic supply to systems is increasing as acidity ameliorates. Effectively, this indicates that some other brake on recovery, besides toxicological stress, must be in action.

One of the three main hypotheses aimed at explaining this brake on widespread biological recovery is that there is a form of internal inertia within previously acid systems, caused by the inherent structure of the food web assemblage formed by the resident community. This inertia could lead, in turn, to lags/hystereses in biological recovery. By focussing on the interactions between species in one such acidified system, I aimed to test this hypothesis, as interactions are widely seen as vital in governing community structure (Allen 1995; Kuhara *et al.* 1999; Montoya *et al.* 2009). While it seems that acid food webs are more dynamically stable and robust at the network level than circumneutral equivalents (Layer *et al.* 2010b; 2013), the underlying mechanisms that confer this stability remain uncertain. It is possible that stability is present at various levels of organisation (individual/population/network), which are not necessarily mutually exclusive from each other. Each level in turn could be conferring stability through individual interactions – both direct and indirect.

The prevalence of Type III functional responses, a quantitative model of interaction strength (Holling 1959), among the resident predator guild is suggestive of this theory at population-level, given that Type III responses are regarded as stabilising, as opposed to the destabilising influence of Type II responses (e.g. Aljetlawai *et al.* 2004; Brose *et al.* 2006b; Dick *et al.* 2013). Predation can play a key role in the trajectory of biological recovery (Murphy *et al.* 2013), and this appears to be the case in this specific system, through density-dependent regulation of prey populations. However, there is evidence that some acid-sensitive species are beginning to persist, albeit at low population densities, despite this widespread response (Woodward & Hildrew 2001). With a lack of evidence for behavioural cues stimulating avoidance mechanisms in either the resident assemblage or potential invaders (at least in this

specific system), the key mechanism that could allow for this persistence, in face of a resistant network structure, might be observed in the context of non-predatory interactions, which leads in turn to indirect interactions between prey species, known as apparent competition. This competition for 'enemy-free space' might prevent populations of colonisers reached high densities, in combination with Type III responses from the predatory guild, but allow for low densities of prey to occasionally persist, despite strong top-down control. By investigating a variety of interactions such as these, a greater understanding of network dynamics become apparent, which allows a more 'whole system' approach to be taken than just analysing food webs from a network-level perspective (Woodward *et al.* 2013).

The stability of acid streams such as Broadstone have so far been characterised by metrics detailing the resilience and robustness of the community (e.g. Layer *et al.* 2010b; 2013) at this network-level, but by examining interactions beneath that level, as work in this thesis shows, an understanding of resistance can also be gained. Inherent resistance manifested through topological properties such interaction strength and patterns align with the 'community closure' hypothesis (Lundberg *et al.* 2003), which suggests niche expansion by residents excludes any potential new colonisers. However, the colonisation of Broadstone by brown trout, as mentioned in Chapter Four, suggests that there are ways of overcoming this community closure and the subsequent resistant food web structure. It could well be that body size, rather than simple toxicological factors like pH tolerance, is the overriding determinant of invasion success. Body size has long been recognised as a significant factor in food web dynamics and stability (e.g. Elton 1927; Peters 1983; Jennings & Mackinson 2003; Woodward *et al.* 2005b). Given that most engulfing predators in Broadstone, and in aquatic systems in general, are gape-limited (Aljetlawi *et al.* 2004; Brose *et al.* 2006a), the size difference between large-bodied colonising predators and the resident predatory guild could facilitate a successive invasion, through 'size refugia' from predation (Chase 1999), as trout

are several orders of magnitude larger than any other predator in the system (Layer *et al.* 2011). There is also the possibility that increased in-stream production is allowing predators to invade.

Secondary production in acidified streams is generally perceived to be low, resulting from a combination of poor algal resources and chemically impaired decomposition (Hildrew *et al.* 1984; Mullholland *et al.* 1992). However, I have shown in Chapter Five that rates of decomposition are increasing as acidity ameliorates. This could in turn lead to greater levels of secondary production, allowing the longer food chains and stronger interactions that are characteristic of trout invasion in headwater streams (Monteith *et al.* 2005; *Layer et al.* 2011), such as those currently observed in Broadstone. This mechanism could allow for the limited recovery thus far observed, as trout invasion creates additional trophic height in the network.

To conclude, freshwater systems are especially vulnerable to anthropogenic stressors (Woodward *et al.* 2013), and this was clearly the case in terms of acidification. While emissions are in almost universal decline across developed industrial nations in North America and Europe (Fowler *et al.* 2005; RotAP 2012; Monteith *et al.* 2013) due to widespread legislative limitations on industry (Stoddard *et al.* 1999; Rose *et al.* 2004), the legacy of anthropogenic acidification remains a major concern in freshwater ecology (Curtis *et al.* 2013). While the benefits of policies curbing emissions can be seen in terms of chemical recovery, there is still a lack of compelling evidence for a corresponding biological recovery.

In this thesis, I have presented evidence that this lag in biological recovery is due, in part, to ecological inertia within acid food webs, governed by direct and indirect interactions at varying levels of organisations. While there is also strong evidence for acidic episodes having a role in delaying recovery in streams (Lepori *et al.* 2003; Kowalik *et al.* 2007; Ormerod &

Durance 2008), these two hypotheses are by no means mutually exclusive. A combination of both could explain why biological recolonisation and persistence of acid-sensitive species is failing to follow the same trajectory as pH increase. There are some suggestions that limited biological recovery is taking place, potentially enabled by increasing in-stream secondary production, which could indicate that a lack of biological recovery is not a static phenomenon and what we are observing, through long-term monitoring programs such as the UKAWMN, are transient dynamics as pH continues to rise. If this is the case, it is vital to continue and increase the scope of such long-term monitoring schemes.

Although these, and other, anthropogenic stressors are seen as grave threats to ecological communities, acidification remains one of the most widespread and significant stressors acting on fresh waters. Predicting the results of such environmental perturbations is a key goal of ecological research (Aufderheide *et al.* 2013). Understanding how identified acid systems respond and recover is vital, if the significance of lasting effects of anthropogenic acidification on freshwaters are to be understood and, most importantly, mitigated.

References

Abjornsson A, Dahl J, Nystrom P, Bronmark C (2001) Influence of predator and dietary chemical cues on the behaviour and shredding efficiency of *Gammarus pulex*. *Aquatic Ecology*, **34**, 379 – 387.

Akaike H (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, **19**, 716-723.

Alewell C, Manderscheid B, Meesenburg H, Bittersohl J (2000) Environmental chemistry: is acidification still an ecological threat? *Nature*, **407**, 856-857.

Aljetlawi AA, Sparrevik E, Leonardsson K (2004). Prey-predator size-dependent functional response: derivation and rescaling to the real world. *Journal of Animal Ecology*, **73**, 239–252.

Allan JD (1982) The effects of reduction in trout density on the invertebrate community of a mountain stream. *Ecology*, **63**, 1444-1455.

Allan JD (1995) *Stream Ecology*. Chapman and Hall, London.

Allen KR (1951) The Horokiwi Stream: A study of a trout population. *New Zealand Marine Department Fish Bulletin*, **10**, 1-231.

Almer B, Dickinson W, Ekstrom C, Hornstrom E, Miller U (1974) Effects of acidification of Swedish lakes. *Ambio*, **3**, 30-36.

Aufderheide H, Rudolf L, Gross T, Lafferty KD (2013) How to predict community responses to perturbations in the face of imperfect knowledge and network complexity. *Proceedings of the Royal Society B: Biological Sciences*, **280**, 2013-2355.

Ayala FJ (1971) Competition between species: frequency dependence. *Science*, **171**, 820-824.

Baglinière JL, Maisse G (1999) *Biology and ecology of the brown and sea trout*. Springer, London.

Baker JP, Schofield CL (1982) Aluminum toxicity to fish in acidic waters. In *Long-Range Transport of Airborne Pollutants* (pp. 289-309). Springer, Netherlands.

Battarbee RW, Flower RJ, Stevenson AC, Jones VJ, Harriman R, Appleby PG (1988) Diatom and chemical evidence for reversibility of acidification of Scottish lochs. *Nature*, **332**, 530-532.

Battarbee RW, Howells G, Skeffington RA, Bradshaw AD (1990) The causes of lake acidification, with special reference to the role of acid deposition. *Philosophical Transactions of the Royal Society B*, **327**, 339-347.

Battarbee RW, Shilland EM, Kernan M, Monteith DT, Curtis CJ (2013). Recovery of acidified surface waters from acidification in the United Kingdom after twenty years of chemical and biological monitoring (1988–2008). *Ecological Indicators* (in press).

Baudoin JM, Guerold F, Felten V, Chauvet E, Wagner P, Rousselle P (2008) Elevated aluminium concentration in acidified headwater streams lowers aquatic hyphomycete diversity and impairs leaf litter breakdown. *Microbial Ecology*, **56**, 260-269

Bechara JA, Moreau G, Hare L (1993) The impact of brook trout (Salvelinus fontinalis) on an experimental stream benthic community: the role of spatial and size refugia. *Journal of Animal Ecology*, **62**, 451-464.

Beckerman AP, Uriarte M, Schmitz OJ (1997) Experimental evidence for behavior-mediated trophic cascade in terrestrial food chain. *Proceedings of the National Academy of Sciences, USA*, **94**, 10735–10738.

Beckerman AP, Petchey OL, Warren PH (2006) Foraging biology predicts food web complexity. *Proceedings of the National Academy of Sciences, USA*, **103**, 13745–13749.

Begon M, Harper JL, Townsend CR (2009) *Ecology: From individuals to ecosystems*. Blackwell, Oxford.

Berlow EL (1999) Strong effects of weak interactions in ecological communities. *Nature*, **398**, 330–333.

Berlow EL, Neutel AM, Cohen JE, de Ruiter PC, Ebenman B, Emmerson MC, Fox JW, Jansen VAA, Jones JI, Kokkoris GD, Logofet DO, McKane AJ, Montoya JM, Petchey O (2004) Interaction strengths in food webs: issues and opportunities. *Journal of Animal Ecology*, **73**, 585–598.

Berlow EL, Dunne JA, Martinez ND, Stark PB, Williams RJ, Brose U (2009) Simple prediction of interaction strengths in complex food webs. *Proceedings of the National Academy of Sciences USA*, **106**, 187-191.

Birks HJB, Line JM, Juggins S, Stevenson AC, ter Braak CJF (1990) Diatoms and pH reconstruction. *Philosophical Transactions of the Royal Society B*, **327**, 263–278.

Bolker B (2008) *Ecological Models and Data in R*. Princeton University Press, Princeton, NJ.

Bonsall MB, Hassell MP (1997) Apparent competition structures ecological assemblages. *Nature*, **388**, 371-373.

Boulton AJ, Quinn JM (2000) A simple and versatile technique for assessing cellulose decomposition potential in floodplain and riverine sediments. *Archiv für Hydrobiologie*, **150**, 133–151.

Bradley DC, Ormerod SJ (2002) Long-term effects of catchment liming on invertebrates in upland streams. *Freshwater Biology*, **47**, 161-171.

Brose U, Jonsson T, Berlow EL, Warren P, Banasek-Richter C, Bersier LF, Blanchard JL, Brey T, Carpenter SR, Blandenier MF, Cushing L, Dawah HA, Dell T, Edwards F, Harper-Smith S, Jacob U, Ledger ME, Martinez ND, Memmott J, Mintenbeck K, Pinnegar JK, Rall BC, Rayner TS, Reuman DC, Liliane R, Ulrich W, Williams RJ, Woodward G, Cohen JE

(2006a) Consumer-resource body-size relationships in natural food webs. *Ecology*, *87*(10), 2411-2417.

Brose U, Williams RJ, Martinez ND (2006) Allometric scaling enhances stability in complex food webs. *Ecology Letters*, **9**, 1228-1236.

Brose U (2010). Body-mass constraints on foraging behaviour determine population and food-web dynamics. *Functional Ecology*, **24**, 28-34.

Brown JH, Gillooly JF (2003) Ecological food webs: High-quality data facilitate theoretical unification. *Proceedings of the Natural Academy of Sciences USA*, **100**, 1467–1468.

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. *Ecology*, **85**, 1771-1789.

Burton, TM, Stanford, RM, Allan, JW (1985) Acidification effects on stream biota and organic matter processing. *Canadian Journal of Fisheries and Aquatic Sciences*, **42**, 669-675

Camerano L (1880) Dell'equilibrio dei viventi mercé la reciproca distruzione. *Atti della Reale Accademia delle Scienze di Torino*, **15**, 393–414.

Chamier, AC (1987) The effect of pH on microbial degradation of leaf litter in the English Lake District. *Oecologia*, **71**, 491-500.

Chaneton EJ, Bonsall MB (2000) Enemy-mediated apparent competition: empirical patterns and the evidence. *Oikos*, **88**, 380–94.

Chase, JM (1999) Food web effects of prey size refugia: variable interactions and alternative stable equilibria. *The American Naturalist*, **154**, 559-570.

Chivers DP, Wisenden BD, Smith JF (1996) Damselfly larvae learn to recognise predators from chemical cues in the predator's diet. *Animal Behaviour*, **52**, 315 – 320.

Christianou M, Ebenman B (2005) Keystone species and vulnerable species in ecological communities: strong or weak interactors? *Journal of Theoretical Biology*, **235**, 95–103.

Clitherow LR, Carrivick JL, Brown LE (2013). Food Web Structure in a Harsh Glacier-Fed River. *PLoS One*, **8**, e60899.

Cohen JE (1978) *Food Webs and Niche Space.* Princeton University Press, Princeton, NJ, USA.

Cohen JE, Jonsson T, Carpenter SR (2003) Ecological community description using the food web, species abundance, and body size. *Proceedings of the National Academy of Sciences of the U.S.A.*, **100**, 1781-1786.

Corbet PS (1999) *Dragonflies: behaviour and ecology of Odonata*. Harley Books, Colchester.

Cowan CA, Peckarsky BL (1994) Diel feeding and positioning periodicity of a grazing mayfly in a trout stream and a fishless stream. *Canadian Journal of Fisheries and Aquatic Sciences*, **51**, 450-459

Crawley MJ (2012) *The R Book*. John Wiley and Sons Ltd, Chichester, UK.

Cummins KW (1974) Structure and function of stream ecosystems. *Bioscience*, **24**, 631-641.

Curtis CJ, Battarbee RW, Monteith DT, Shilland EM (2013) The future of upland water ecosystems of the UK in the 21st Century: A synthesis. *Ecological Indicators* (in press).

Damuth J (1981) Population density and body size in mammals. *Nature*, **290**, 699–700.

Dangles O, Guerold F (2001) Influence of shredders in mediating breakdown rates of beech leaves in circumneutral and acidic forest streams. *Archiv für Hydrobiologie*, **151**, 649-666.

Dangles O, Gessner MO, Guerold F, Chauvet E (2004) Impact of stream acidification on litter breakdown: Implications for assessing ecosystem functioning. *Journal of Applied Ecology*, **41**, 365-378.

Darwin C (1859) *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. John Murray, London.

Davies L, Bates JW, Bell JNB, James PW, Purvis OW (2007) Diversity and sensitivity of epiphytes to oxides of nitrogen in London. *Environmental Pollution*, **146**, 299-310.

De Ruiter PC, Neutel AM, Moore JC (1995). Energetics, patterns of interaction strengths, and stability in real ecosystems. *Science*, **269**, 1257-1260.

De Wit CT (1960) On competition. *Versl. landbouwk. underz.*, **66**, 8.

Didham RK, Blakely TJ, Ewers RM, Hitchings TR, Ward JB, Winterbourn MJ (2012) Horizontal and vertical structuring in the dispersal of adult aquatic insects in a fragmented landscape. *Fundamental and Applied Limnology/Archiv für Hydrobiologie*, **180**, 27-40

Dobson M, Hildrew AG (1992). A test of resource limitation among shredding detritivores in low order streams in southern England. *Journal of Animal Ecology*, **61**, 69-77.

Driscoll CT, Lawrence GB, Bulger AJ, Butler TJ, Cronan CS, Eagar C, Lambert KF, Likens GE, Stoddard JL, Weathers KC (2001) Acidic deposition in the northeastern United States: Sources and inputs, ecosystem effects, and management strategies. *Bioscience*, **51**, 180–198.

Driscoll CT, Driscoll KM, Roy KM, Dukett J (2007) Changes in the chemistry of lakes in the Adirondack region of New York following declines in acidic deposition. *Applied Geochemistry*, **22**, 1181–1188.

Egglishaw HJ (1972) An experimental study of the breakdown of cellulose in fast-flowing streams. *Memorie dell'Istituto Italiano di Idrobiologia (Supplement),* **29,** 408-428.

Elton CS (1927) *Animal Ecology*. Sedgewick and Jackson, London.

Emmerson MC, Raffaelli D (2004). Predator–prey body size, interaction strength and the stability of a real food web. *Journal of Animal Ecology*, **73**, 399–409.

Emmerson MC, Montoya JM, Woodward G (2005) Body size, interaction strength and food web dynamics. In: *Dynamic Food Webs: Multispecies Assemblages, Ecosystem Development and Environmental Change* (Eds P. de Ruiter, V. Wolters, & J.C. Moore), pp. 167-178. Academic Press: Theoretical Ecology Series.

Fagan WF, Hurd LE (1994) Hatch density variation of a generalist arthropod predator – population consequences and community impact. *Ecology*, **75**, 2022–2032.

Feeley HB, Kerrigan C, Fanning P, Hannigan E, Kelly-Quinn M (2011) Longitudinal extent of acidification effects of plantation forest on benthic macroinvertebrate communities in soft water streams: evidence for localised impact and temporal ecological recovery. *Hydrobiologia*, **671**, 217-226.

Feeley HB, Bruen M, Blacklocke S, Kelly-Quinn M (2013) A regional examination of episodic acidification response to reduced acidic deposition and the influence of plantation forests in Irish headwater streams. *Science of the Total Environment*, **443**, 173-183.

Fernandes I, Pascoal C, Guimarães HA, Pinto R, Sousa I, Cássio F (2012) Higher temperature reduces the effect of litter quality on decomposition by aquatic fungi. *Freshwater Biology*, **57**, 2306-2317.

Ferreira V, Chauvet E (2011) Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. *Global Change Biology*, **17**, 551-564.

Fincke OM, Yanoviak SP, Hanschu RD (1997) Predation by odonates depresses mosquito abundance in water-filled tree holes in Panama. *Oecologia*, **112**, 244-253.

Flecker AS, Townsend CR (1994) Community-wide consequences of trout introduction in New Zealand streams. *Ecological Applications*, **4**,798-807.

Flower RJ, Battarbee RW (1983) Diatom evidence for recent acidification of two Scottish lochs. *Nature*, **305**, 130-133.

Forbes SA (1887) The lake as a microcosm. *Bulletin of the Peoria Scientific Association*, **7**, 7–87.

Fowler D, Smith RI, Muller JBA, Hayman G, Vincent KJ (2005) Changes in the atmospheric deposition of acidifying compounds in the UK between 1986 and 2001. *Environmental Pollution*, **137**, 15-25.

Fransen B, de Kroon H, Berendse F (2001). Soil nutrient heterogeneity alters competition between two perennial grass species. *Ecology*, **82**, 2534-2546.

Fromm PO (1980) A review of some physiological and toxicological responses of freshwater fish to acid stress. *Environmental Biology of fishes*, **5**, 79-93.

Futuyma DJ (1970) Variation in genetic response to interspecific competition in laboratory populations of *Drosphilia*. *American Naturalist*, **104**, 239-252.

Gorham E (1955) On the acidity and salinity of rain. *Geochimica et Cosmochimica Acta*, **7**, 231-239.

Gorham E (1998) Acid deposition and its ecological effects: a brief history of research. *Environmental Science and Policy*, **1**, 153-166.

Grabowski M, Bacela K, Konopacka A (2007). How to be an invasive gammarid (Amphipoda: Gammaroidea)–comparison of life history traits. *Hydrobiologia*, **590**, 75-84.

Griffith MB, Perry SA (1993) The distribution of macroinvertebrates in the hyporheic zone of two small Appalachian headwater streams. *Archiv für Hydrobiologie*, **126**, 373-384.

Groom AP, Hildrew AG (1989) Food quality for detritivores in streams of contrasting pH. *Journal of Animal Ecology*, **58**, 863-881.

Guo JH, Liu XJ, Zhang Y, Shen JL, Han WX, Zhang WF, Christie P, Goulding KWT, Vitousek PM, Zhang FS (2010) Significant acidification in major Chinese croplands. *Science*, **327**, 1008-1010.

Hall SJ, Raffaelli D (1993) Food webs: theory and reality. *Advances in Ecological Research*, **24**, 187-239.

Hämäläinen H, Huttunen P (1996) Inferring the minimum pH of streams from macroinvertebrates using weighted averaging regression and calibration. *Freshwater Biology*, **36**, 697–709.

Hargeby A (1990) Effects of pH, humic substances and animal interactions on survival and physiological status of *Asellus aquaticus* L. and *Gammarus pulex* (L.). *Oecologia*, **82**, 348-354.

Hargeby A, Petersen RC (1988) Effects of low pH and humus on the survivorship, growth and feeding of *Gammarus pulex* (L.)(Amphipoda). *Freshwater biology*, **19**, 235-247.

Harvey HH (1980) Widespread and diverse changes in the biota of North American lakes and rivers coincident with acidification. In *Proceedings of an International Conference on the Ecological Impact of Acid Precipitation* (Eds. D. Drablos, A. Tollan), pp. 93-98, Sandefjord, Norway.

Hettelingh JP, Posch M, Slootweg J. (2008) Critical Load, Dynamic Modelling and Impact Assessment in Europe: CCE Status Report 2008. Report No 500090003. Coordination Centre for Effects, Netherlands Environmental Assessment Agency.

Hildrew AG (1992) Food webs and species interactions. In: *The Rivers Handbook* (Eds: P Calow, GE Petts), pp 309-330. Blackwell Scientific Publications, Oxford, UK.

Hildrew AG (2009) Sustained Research on Stream Communities: a model system and the comparative approach. *Advances in Ecological Research*, **41,** 175-312.

Hildrew AG, Townsend CR (1977) The influence of substrate on the functional response of Plectrocnemia conspersa (Curtis) larvae (Trichoptera: Polycentropodidae). *Oecologia*, **31**, 21-26.

Hildrew AG, Townsend CR, Francis J, Finch K (1984) Cellulolytic decomposition in streams of contrasting pH and its relationship with invertebrate community structure. *Freshwater Biology*, **14**, 323-328

Hildrew AG, Townsend CR, Hasham A (1985) The predatory Chironomidae of an iron-rich stream: feeding ecology and food web structure. *Ecological Entomology*, **10**, 403–413.

Hildrew AG, Ormerod SJ (1995) Acidification: causes, consequences and solutions. In: *The Ecological Basis for River Management* (Ed. by D. M. Harper and A. J. D. Ferguson), pp. 147-160. John Wiley and Sons Ltd, London.

Hildrew AG, Woodward G, Winterbottom JH, Orton S (2004) Strong density dependence in a predatory insect: larger scale experiments in a stream. *Journal of Animal Ecology*, **73**, 448-458.

Hildrew AG, Raffaelli DG, Edmonds-Brown R (Eds.) (2007) *Body size: the structure and function of aquatic ecosystems*. Cambridge University Press.

Hladyz S, Gessner MO, Giller PS, Pozo J, Woodward G (2009) Resource quality and stoichiometric constraints on stream ecosystem functioning. *Freshwater Biology*, **54**, 957–97.

Hobbie SE, Eddy WC, Buyarski CR, Carol Adair E, Ogdahl ML, Weisenhorn P (2012) Response of decomposing litter and its microbial community to multiple forms of nitrogen enrichment. *Ecological Monographs*, **82**, 389-405.

Holling C (1959) Some characteristics of simple types of predation and parasitism. *The Canadian Entomologist*, **91**, 385–398.

Holt RD (1977). Predation, apparent competition, and the structure of prey communities. *Theoretical Population Biology*, **12**, 197-229.

Holt RD, Lawton JH (1993) Apparent competition and enemy-free space in insect host-parasitoid communities. *American Naturalist*, **142**, 623-45.

Holt RA, Lawton JH (1994) The ecological consequences of shared natural enemies. *Annual Review of Ecology and Systematics*, **25**, 495-520.

Huang C, Sih A (1991) Experimental studies on direct and indirect interactions in a three trophic-level stream system. *Oecologia*, 85, 530-536.

Huryn AD (1996) An appraisal of the Allen paradox in a New Zealand trout stream. *Limnology and Oceanography*, **41**, 243-252.

Huxley JS (1932) *Problems of relative growth*. Methuen, London.

Imberger SJ, Thompson RM, Grace MR (2010) Searching for effective indicators of ecosystem function in urban streams: assessing cellulose decomposition potential. *Freshwater Biology*, **55**, 2089-2106.

Ings TC, Montoya JM, Bascompte J, Bluthgen N, Brown L, Dormann CF, Edwards F, Figueroa D, Jacob U, Jones JI, Lauridsen RB, Ledger ME, Lewis HM, Oleson JM, van Veen, FJL, Warren PH, Woodward G (2009) Ecological networks – beyond food webs. *Journal of Animal Ecology*, **78**, 253 – 269.

Inouye RS, Schaffer WM (1981) On the ecological meaning of ratio (de Wit) diagrams in plant ecology. *Ecology*, **62**, 1679-1681.

Jacob U, Thierry A, Brose U, Arntz WE, Berg S, Brey T, Fetzer I, Jonsson T, Mintenbeck K, Mollman C, Petchey OL, Riede JO, Dunne JA (2011) The role of body size in complex food webs: A cold case. *Advances In Ecological Research*, **45**, 181-223.

Jeffries MJ, Lawton JH (1984). Enemy free space and the structure of ecological communities. *Biological Journal of the Linnean Society*, **23**, 269-286.

Jenkins GB, Woodward G, Hildrew AG (2013) Long-term amelioration of acidity accelerates decomposition in headwater streams, *Global Change Biology*, **19**, 1100-1106.

Jennings S, Mackinson S (2003) Abundance-body mass relationships in size-structured food webs. *Ecology Letters*, **6,** 971-974.

Jensen KW, Snekvik E (1972) Low pH levels wipe out salmon and trout populations in southernmost Norway. *Ambio*, **1**, 223-225.

Jeschke JM, Kopp M, Tollrain R (2004) Consumer-food systems: why type I functional responses are exclusive to filter feeders. *Biological Reviews*, **79**, 337-349

Johnson RK, Angeler DG (2010) Tracing recovery under changing climate: response of phytoplankton and invertebrate assemblages to decreased acidification. *Journal of the North American Benthological Society*, **29**, 1472-1490.

Junge CO, Libosvarsky J (1965). Effects of size selectivity on population estimates based on successive removals with electrical fishing gear. *Zool. listy*,**14**, 171-178.

Kalinkat G, Rall BC, Vucic-Pestic O, Brose U (2011) The allometry of prey preferences. *PLoS One*, **6**, 1-11.

Kampfraath AA, Hunting ER, Mulder C, Breure AM, Gessner MO, Kraak MHS, Admiraal W (2012) DECOTAB: a multipurpose standard substrate to assess effects of litter quality on microbial decomposition and invertebrate consumption. *Freshwater Science*, **31**, 1156–1162.

Kéfi S, Berlow EL, Wieters EA, Navarrete SA, Petchey OL, Wood SA, Boit A, Joppa JN, Lafferty KD, Williams RJ, Martinez ND, Menge BA, Blanchette CA, Iles AD, Brose U (2012). More than a meal…integrating non-feeding interactions into food webs. *Ecology Letters*, **15**, 291-300.

Kohler SL, Wiley MJ (1997). Pathogen outbreaks reveal large-scale effects of competition in stream communities. *Ecology*, **78**, 2164-2176.

Kowalik RA, Ormerod SJ (2006) Intensive sampling and transplantation experiments reveal continued effects of episodic acidification on sensitive stream invertebrates. *Freshwater Biology*, **51**, 180-191.

Kowalik RA, Cooper DM, Evans CM, Ormerod SJ (2007) Acid episodes retard the biological recovery of upland British streams from acidification. *Global Change Biology*, **13**, 2439-2452.

Knight TM, McCoy MW, Chase JM, McCoy KA, Holt RD (2005) Trophic cascades across ecosystems. *Nature*, **437**, 880-883.

Krueger CC, Waters TF (1983) Annual production of macroinvertebrates in three streams of different water quality. *Ecology*, **64**, 840-850.

Kuhara N, Nakano S, Miyasaka H (1999) Interspecific competition between two stream insect grazers mediated by non-feeding predatory fish. *Oikos*, **87**, 27-35.

Kuzniar K (1948) Studies on the cellulose decomposition in forest soil. *Instytut Badawczy Lesnictwa. Seria A: Rozprawy i Sprawozdania,* **50**, 1-44.

Lacoul P, Freedman B, Clair T (2011). Effects of acidification on aquatic biota in Atlantic Canada. *Environmental Reviews*, **19**, 429-460.

Lancaster J (1988) *Invertebrate predation and community structure in an acid stream*. PhD Thesis, Queen Mary & Westfield College.

Lancaster J, Hildrew AG, Townsend CR (1991) Invertebrate predation on patchy and mobile prey in streams. *Journal of Animal Ecology*, **60**, 625-641.

Lancaster J, Downes BJ, Arnold A (2010). Environmental constraints on oviposition limit egg supply of a stream insect at multiple scales. *Oecologia*, **163**, 373-384.

Lang B, Rall BC, Brose U (2012). Warming effects on consumption and intraspecific interference competition depend on predator metabolism. *Journal of Animal Ecology*, **81**, 516-523.

Larrañaga A, Layer K, Basaguren A, Pozo J, Woodward G (2010) Consumer body composition and community structure in a stream is altered by pH. *Freshwater Biology*, **55**, 670-680.

Latter PM, Howson G (1977) The use of cotton strips in indicate cellulose decomposition in the field. *Pedobiologia*, **17**, 145-155.

Latter PM, Walton DWH (1988) The cotton strip assay for cellulose decomposition studies in soil: history of the assay and development. In: *Cotton Strip Assay: An Indicator of Decomposition* (Eds A.F. Harrison, P.M. Latter & D.W.H. Walton), pp 7-10. Institute of Terrestrial Ecology, Grange-over-Sands.

Lawton JH (1986) The effect of parasitoids on phytophagous insect communities. In: *Insect Parasitoids - 13th Symposium of the Royal Entomological Society, London* (Eds J Waage & D Greathead), pp 265-87. London Academic.

Layer K (2010). *Responses of freshwater food webs to spatial and temporal pH gradients*. PhD Thesis, Queen Mary & Westfield College, University of London, 236pp.

Layer K, Hildrew AG, Monteith D, Woodward G (2010a) Long-term variation in the littoral food web of an acidified mountain lake. *Global Change Biology*, **16**, 3133-3143.

Layer K, Riede J, Hildrew AG, Woodward G (2010b) Food web structure and stability in 20 streams across a wide pH gradient. *Advances in Ecological Research*, **42**, 265-299.

Layer K, Hildrew AG, Jenkins GB, Riede JO, Rossiter SJ, Townsend CR, Woodward G (2011) Long-term dynamics of a well-characterized food web: four decades of acidification and recovery in the Broadstone Stream model system. *Advances in Ecological Research,* **44**, 69-117.

Layer K, Hildrew AG, Woodward G (2013) Grazing and detritivory in 20 stream food webs across a broad pH gradient. *Oecologia*, **171**, 459-471.

Ledger ME, Hildrew AG (2000) Herbivory in an acid stream. *Freshwater Biology*, **43**, 545-556.

Ledger ME, Hildrew AG (2000). Resource depression by a trophic generalist in an acid stream. *Oikos,* **90**, 271-278.

Ledger ME, Hildrew AG (2001) Recolonization by the benthos of an acid stream following a drought. *Archiv für Hydrobiologie,* **152**, 1-17.

Ledger ME, Hildrew AG (2005) The ecology of acidification and recovery: changes in herbivore-algal food web linkages across a pH gradient in streams. *Environmental Pollution,* **137**, 103-118.

Ledger ME, Brown LE, Edwards FK, Milner AM, Woodward G (2012). Drought alters the structure and functioning of complex food webs. *Nature Climate Change*, **3**, 223-227.

Loeuille N, Loreau M. (2004) Nutrient enrichment and food chains: can evolution buffer top-down control? *Theoretical Population Biology*, **65**, 285-298.

Lundberg P, Ranta E, Kaitala V (2000) Species loss leads to community closure. *Ecology Letters*, **3**, 465–46.

Luttbeg B, Kerby JL (2005) Are scared prey as good as dead? *Trends in Ecology & Evolution*, **20**, 416–418.

Lynch JA, Bowersox VC, Grimm JW (2000) Changes in sulfate deposition in eastern USA following implementation of Phase I of Title IV of the Clean Air Act Amendments of 1990. *Atmospheric Environment*, **34**, 1665-1680.

MacArthur R (1955) Fluctuations of animal populations and measure of community stability. *Ecology*, **36**, 533–536.

MacNeale KH, Peckarsky BL, Likens GE (2005) Stable isotopes identify dispersal patterns of stonefly populations living along stream corridors. *Freshwater Biology*, **50**, 1117–1130.

Mason CF (1991) *Biology of freshwater pollution*, 2nd edn. Longman Scientific & Technical, New York, USA.

Masters Z, Peteresen I, Hildrew AG, Ormerod SJ (2007) Insect dispersal does not limit the biological recovery of streams from acidification. *Aquatic Conservation: Marine & Freshwater Ecosystems* **17**, 375-383.

May RM (1972) Will a large complex system be stable? *Nature*, **238**, 413-414

May RM (1973) *Stability and Complexity in Modern Ecosystems*. Princeton University Press, Princeton, New Jersey.

McAuliffe JR (1984). Competition for space, disturbance, and the structure of a benthic stream community. *Ecology*, 65, 894-908

McCann KS (2000) The diversity–stability debate. *Nature*, **405**, 228-233.

McCann KS, Hastings A, Huxel GR (1998) Weak trophic interactions and the balance of nature. *Nature*, **395**, 794-797.

McCann KS, Rasmussen JB, Umbanhowar J (2005) The dynamics of spatially coupled food webs. *Ecology Letters*, **8**, 513–523.

McKie BG, Petrin Z, Malmqvist B (2006) Mitigation or disturbance? Effects of liming on macroinvertebrate assemblage structure and leaf-litter decomposition in the humic streams of northern Sweden. *Journal of Applied Ecology*, **43**, 780-791.

McIntosh AR, Peckarsky BL (1996) Differential behavioural responses of mayflies from streams with and without fish to trout odour. *Freshwater Biology*, **35**, 141-148.

McIntosh AR, Peckarsky BL (1999). Criteria determining behavioural responses to multiple predators by a stream mayfly. *Oikos*, 85, 554-564.

McPeek MA (1998) The consequences of changing the top predator in a food web: a comparative experimental approach. *Ecological Monographs*, **68**, 1-23.

McPeek MA, Peckarsky BL (1998) Life histories and the strengths of species interactions: combining mortality, growth, and fecundity effects. *Ecology*, **79**, 867-879.

Menge BA (1995) Indirect effects in marine rocky intertidal interaction webs: patterns and importance. *Ecological Monographs*, **65**, 21-74.

Merrix FL, Lewis BR, Ormerod SJ (2006) The effect of low pH and palliative liming on beech litter decomposition in acid-sensitive streams. *Hydrobiologia*, **571**, 373-381.

Mithen SJ, Lawton JH (1986) Food-web models that generate constant predator-prey ratios. *Oecologia*, **69**, 542-550.

Monteith DT, Evans CD (Eds.) (2000). The UK Acid Waters Monitoring Network: Ten Year Report. Analysis and Interpretation of Results, 1988-1998. ENSIS Publishing, London. 363 pp.

Monteith DT, Hildrew AG, Flower RJ, Raven PJ, Beaumont WRB, Collen P, Krieser AM, Shilland EM, Winterbottom JH (2005) Biological responses to the chemical recovery of acidified fresh waters in the UK. *Environmental Pollution*, **137**, 83-101.

Monteith DT, Evans CD, Henrys PA, Simpson GL, Malcolm IA (2013) Trends in the hydrochemistry of acid-sensitive surface waters in the UK 1988–2008. *Ecological Indicators* (in press)

Montoya JM, Woodward G, Emmerson MC, Sole RV (2009) Press perturbations and indirect effects in real food webs. *Ecology*, **90**, 2426-2433.

Mulholland PJ, Elwood JW, Palumbo PV, Stevenson RJ (1986) Effect of stream acidification on periphyton composition, chlorophyll, and productivity. *Canadian Journal of Fisheries and Aquatic Sciences*, **43**, 1846-1858.

Mulholland PJ, Driscoll CT, Elwood JW, Osgood MP, Paulmbo AV, Rosemund AD, Smith ME, Schofield C (1992) Relationships between stream acidity and bacteria, macroinvertebrates, and fish: a comparison of north temperate and south temperate mountain streams, USA. *Hydrobiologia*, **239**, 7-24.

Müller CB, Godfray HCJ (1997) Apparent competition between two aphid species. *Journal of Animal Ecology*, **66**, 57–64.

Murdoch WW, Stewart-Oaten A (1975) Predation and population stability. *Advances in Ecological Research*, **9**, 2–13.

Murphy JF, Winterbottom JH, Orton S, Simpson GL, Shilland EM, Hildrew AG (2013) Evidence of recovery from acidification in the macroinvertebrate assemblages of UK fresh waters: a 20-year time series. *Ecological Indicators* (in press).

Nakano S, Miyasaka H, Kuhara N (1999). Terrestrial-aquatic linkages: Riparian arthropod inputs alter trophic cascades in a stream food web. *Ecology*, **80**, 2435–2441.

Neutel AM, Heesterbeek JAP, de Ruiter PC (2002) Stability in real food webs: weak links in long loops. *Science*, **296**, 1120–1123.

Niyogi DK, McKnight DM, Lewis Jr WM (2002) Fungal communities and biomass in mountain streams affected by mine drainage. *Archiv für Hydrobiologie*, **155**, 255-271.

O'Gorman EJ, Emmerson MC (2009) Perturbations to trophic interactions and the stability of complex food webs. *Proceedings of the Natural Academy of Sciences of the U.S.A.*, **106**, 13393-13398.

O'Gorman EJ, Jacob U, Jonsson T, Emmerson MC (2010) Interaction strength, food web topology and the relative importance of species in food webs. *Journal of Animal Ecology*, **79,** 682-692.

O'Gorman EJ, Pichler DE, Adams G, Benstead JP, Cohen H, Craig N, Cross WF, Demars BOL, Nikolai F, Gislason G, Gudmundsdottir R, Hawcsak A, Hood JM, Hudson LN, Johansson LS, Johnasson MP, Junker JR, Laurila A, Manson JR, Mavromati E, Nelson D, Olafsson J, Perkins DM, Petchey OL, Reuman DC, Rall BC, Stewart R, Thompson MSA, Woodward G (2012). Impacts of warming on the structure and functioning of aquatic communities: individual-to ecosystem-level responses. *Advances in Ecological Research*, **47**, 81-176.

Olesen JM, Dupont YL, O'Gorman EJ, Ings TC, Layer K, Melian CJ, Trojelsgaard K, Pichler DE, Rasmussen C, Woodward G (2010) From Broadstone to Zackenberg: space, time and hierarchies in ecological networks. *Advances in Ecological Research*, **42**, 1–69.

Ormerod SJ, Durance I (2008) Restoration and recovery from acidification in upland Welsh streams over 25 years. *Journal of Applied Ecology*, **46**, 164-174.

Ormerod SJ, Weatherley NS, Varallo PV, Whitehead P (1988) Preliminary empirical records of the historical and future impacts of acidification on the ecology of Welsh streams. *Freshwater Biology*, **20**, 127–140.

Paine RT (1992) Food-web analysis through field measurement of per capita interaction strength. *Nature*, **355**, 73–75.

Pawar S, Dell AI, Savage VM (2012) Dimensionality of consumer search space drives trophic interaction strengths. *Nature*, **486**, 485-489.

Peckarsky BL (1996) Alternative predator avoidance syndromes of stream-dwelling mayfly larvae. *Ecology*, **77**, 1888-1905.

Peckarsky BL, McIntosh AR (1998) Fitness and community consequences of avoiding multiple predators. *Oecologia*, **113**, 565-576.

Peckarsky BL, Abrams PA, Bolnick DI, Dill LM, Grabowski JH, Luttbeg B, Orrock JL, Peacor SD, Pressier EL, Schmitz OJ, Trussell GC (2008) Revisiting the classics: considering nonconsumptive effects in textbook examples of predator-prey interactions. *Ecology*, **89**, 2416-2425.

Petchey OL, Beckerman AP, Riede JO, Warren PH (2008) Size, foraging, and food web structure. *Proceedings of the National Academy of Sciences*, **105**, 4191-4196.

Petchey OL, Brose U, Rall BC (2010) Predicting the effects of temperature on food web connectance. *Philosophical Transactions of the Royal Society B*, **365**, 2081–2091.

Peters RH (1983). *The ecological implications of body size*. Cambridge University Press.

Petersen I, Masters Z, Hildrew AG, Ormerod SJ (2004) Dispersal of adult insects in catchments of differing land use. *Journal of Applied Ecology*, **41**, 934-950.

Petersen RC, Cummins KW (1974) Leaf processing in a woodland stream. *Freshwater Biology*, **4**, 343-368.

Pimm SL (1980) Properties of food webs. *Ecology*, **61**, 219 – 225.

Pimm SL, Lawton JH, Cohen JE (1991) Food web patterns and their consequences. *Nature*, **350**, 669-674.

Polis GA (1998) Stability is woven by complex webs. *Nature*, **395**, 744-745.

Post DM (2002) The long and short of food chain length. *Trends in Ecology and Evolution*, **17**, 269–277.

Preisser EL, Bolnick DI, Benard MF (2005) Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology*, **86**, 501-509.

Pretty JL, Giberson DJ, Dobson M (2005) Resource dynamics and detritivore production in an acid stream. *Freshwater Biology*, **50**, 578-591.

Pritchard G (1965) Prey capture by dragonfly larvae (Odonata; Anisoptera). *Canadian Journal of Zoology*, **43**, 271-289.

Pye MC, Vaughan IP, Ormerod SJ (2012) Episodic acidification affects the breakdown and invertebrate colonisation of oak litter. *Freshwater Biology*, **57**, 2318-2329.

R Development Core Team (2010) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Raddum GG, Fjellheim A (1984) Acidification and early warning organisms in freshwater in western Norway. *Verhandlungen der Internationale Vereinigung Fur Theoretische und Angewandte Limnologie.*, **22**, 1973–1980.

Raddum GG, Fjellheim A (2003) Liming of River Audna, Southern Norway: A large-scale experiment of benthic invertebrate recovery. *Ambio*, **32**, 230-234.

Rall B, Guill C, Brose U (2008) Food-web connectance and predator interference dampen the paradox of enrichment. *Oikos*, **117**, 202-213.

Rall BC, Vucic-Pestic O, Ehnes RB, Emmerson MC, Brose U (2010) Temperature, predator–prey interaction strength and population stability. *Global Change Biology*, **16**, 2145-2157.

Rall BC, Kalinkat G, Ott D, Vucic-Pestic O, Brose U (2011) Taxonomic versus allometric constraints on non-linear interaction strengths. *Oikos*, **120**, 483-492.

Reuman DC, Mulder C, Banasek-Richter C, Blandenier MFC, Breure AM, Den Hollander H, Kneitel JM, Raffaeli D, Woodward G, Cohen JE (2009) Allometry of body size and abundance in 166 food webs. *Advances in Ecological Research*, **41**, 1-44.

Rodríguez DJ (1997) A method to study competition dynamics using de Wit replacement series experiments. *Oikos*, **78**, 411-415.

Rogers D (1972) Random search and insect population models. *Journal of Animal Ecology*, **41**, 369–383.

Rose N, Monteith D, Kettle H, Thompson R, Yang H, Muir D (2004). A consideration of potential confounding factors limiting chemical and biological recovery at Lochnagar, a remote mountain loch in Scotland. *Journal of Limnology*, **63**, 63-76.

Rosemond AD, Reice SR, Elwood JW, Mulholland PJ (1992). The effects of stream acidity on benthic invertebrate communities in the south-eastern United States. *Freshwater Biology*,

27, 193–209.

Rosenqvist IT (1978). Acid precipitation and other possible sources for acidification of rivers and lakes. *Science of the Total Environment*, **10**, 271-272.

RoTAP (2012) Review of Transboundary Air Pollution: Acidification, Eutrophication, Ground Level Ozone and Heavy Metals in the UK. Contract Report to the Department for Environment, Food and Rural Affairs. Centre for Ecology & Hydrology.

Rundle SD, Weatherley NS, Ormerod SJ (1995) The effects of catchment liming on the chemistry and biology of upland Welsh streams: testing model predictions. *Freshwater Biology*, **34**, 165-175.

Sarnelle O, Wilson AE (2008) Type III function response in Daphnia. *Ecology*, **89**, 1723-1732.

Schindler DW (1988) Effects of acid rain on freshwater ecosystems. *Science*, **239**, 149-157.

Schmid-Araya JM, Hildrew AG, Robertson A, Schmid PE, Winterbottom JH (2002) The importance of meiofauna in food webs: Evidence from an acid stream. *Ecology*, **83**, 1271-1285.

Schmitt RJ (1987) Indirect interactions between prey: apparent competition, predator aggregation, and habitat segregation. *Ecology*, **68**, 1887-1897.

Schmitz OJ, Beckerman AP, O'Brien KM (1997) Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. *Ecology*, **78**, 1388–1399.

Schofield K, Townsend CR, Hildrew AG (1988) Predation and the prey community of a headwater stream. *Freshwater Biology*, **20**, 85-95.

Seip HM., Aagaard P, Angell V, Eilertsen O, Larssen T, Lydersen E, Mulder J, Muniz IP, Semb A, Dagang T, Vogt RD, Jinshong X, Jiling X, Dawei Z, Guohui K (1999) Acidification in China: assessment based on studies at forested sites from Chongqing to Guangzhou. *Ambio*, **28**, 522-528.

Sentis A, Hemptinne JL, Brodeur J (2013) Parsing handling time into its components: implications for responses to a temperature gradient. *Ecology*, **94**, 1675-1680.

Sih A (1986) Antipredator responses and the perception of danger by mosquito larvae. *Ecology*, **67**, 434–441.

Sih A (1993) Effects of ecological interactions on forager diets: competition, predation risk, parasitism and prey behaviour. *Diet Selection: An Interdisciplinary Approach to Foraging Behaviour* (ed. R.N. Hughes), pp. 182–211. Blackwell Scientific Publications, Oxford, UK.

Sih A, Englund G, Wooster D (1998) Emergent impacts of multiple predators on prey. *Trends in Ecology & Evolution*, **13**, 350-355.

Simon KS, Townsend CR (2003) Impacts of freshwater invaders at different levels of

ecological organisation, with emphasis on salmonid and ecosystem consequences. *Freshwater Biology*, **49**, 982-944.

Simon KS, Simon MA, Benfield EF (2009) Variation in ecosystem function in Appalachian streams along an acidity gradient. *Ecological Applications*, **19**, 1147-1160.

Simon KS, Chadwick MA, Huryn AD, Valett HM (2010) Stream ecosystem response to chronic deposition of N and acid at the Bear Brook Watershed, Maine. *Environmental Monitoring and Assessment*, **171**, 83-92.

Skjelkvåle BL, Stoddard JL, Jeffries DS, Tørseth K, Høgasen T, Bowman J, Mannio J, Monteith DT, Mosello R, Rogora M, Rzychon D, Vesely J, Wieting J, Wilander A, Worsztynowicz A (2005) Regional scale evidence for improvements in surface water chemistry 1990-2001. *Environmental Pollution*, **137**, 165-176.

Speirs DC, Gurney WSC, Winterbottom JH, Hildrew AG (2000) Long-term demographic balance in the Broadstone Stream insect community. *Journal of Animal Ecology*, **69**, 45-58.

Stead TK, Schmid-Araya JM, Hildrew AG (2003). All creatures great and small: patterns in the stream benthos across a wide range of metazoan body size. *Freshwater Biology*, **48**, 532-547.

Stead TK, Schmid-Araya JM, Hildrew AG (2005) Secondary production of a stream metazoan community: Does the meiofauna make a difference? *Limnology & Oceanography*, **50**, 398-403.

Stoddard JL, Jeffries DS, Lükewille A, Clair TA, Dillon PJ, Driscoll CT, Forsius M, Johannessen M, Kahl JS, Kellogg JH, Kemp A, Mannio J, Monteith DT, Murdoch PS, Patrick S, Rebsdorf A, Skjelkvåle BL, Stainton MP, Traaen T, van Dam H, Webster KE, Wieting J, Wilander, A (1999) Regional trends in aquatic recovery from acidification in North America and Europe. *Nature*, **401**, 575-578.

Suberkropp K, Chauvet E (1995) Regulation of leaf breakdown by fungi in streams: influences of water chemistry. *Ecology*, **76**, 1433–1445.

Sutcliffe DW, Carrick TR (1973) Studies on mountain streams in the English Lake District. *Freshwater Biology*, **3**, 437–462.

Sutcliffe DW, Hildrew AG (1989) Invertebrate communities in acid streams. In *Acid Toxicity and Aquatic Animals* (Eds R. Morris, E.W. Taylor, D.J.A. Brown & J.A. Brown). Seminar Series of the Society for Experimental Biology, Cambridge University Press, 13-29.

Thompson RM, Brose U, Dunne JA, Hall Jr RO, Hladyz S, Kitching RL, Martinez DM, Rantala H, Romanuk TM, Stouffer DB, Tylianakis JM (2012). Food webs: reconciling the structure and function of biodiversity. *Trends in Ecology and Evolution*, **27**, 698-697.

Tiegs SD, Langhans SD, Tockner K, Gessner MO (2007) Cotton strips as a leaf surrogate to measure decomposition in river flood plain habitats. *Journal of the North American Benthological Society*, **26**, 70-77.

Tilman D, Downing JA (1994) Biodiversity and stability in grasslands. *Nature,* **367,** 363–365.

Tilman D, Wedin D, Knops J (1996) Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature*, **379**, 718-720.

Tipping E, Bass JAB, Hardie D, Haworth EY, Hurley MA, Wills G (2002) Biological responses to the reversal of acidification in surface waters of the English Lake District. *Environmental Pollution*, **116**, 137-146.

Townsend CR (1996) Invasion biology and ecological impacts of brown trout *Salmo trutta* in New Zealand. *Biological Conservation*, **78**, 13-22.

Townsend CR, Hildrew AG (1976) Field experiments on the drifting, colonization and continuous redistribution of stream benthos. *Journal of Animal Ecology*, **45**, 759-772.

Townsend CR, Hildrew AG (1979) Form and function in the prey-catching net of *Plectrocnemia conspersa* (Trichoptera: Polycentropodidae). *Oikos,* **33**, 412–418.

Townsend CR, Simon KS (2006) Consequences of brown trout invasion for stream ecosystems. In: *Biological Invasions in New Zealand* (Ed. by R.B. Allen and W.G. Lee), pp. 213–225. Springer Verlag, Berlin.

Townsend CR, Hildrew AG, Francis JE (1983) Community structure in some Southern English streams: the influence of physicochemical factors. *Freshwater Biology*, **13**, 531-544.

Townsend CR, Hildrew AG, Schofield K (1987) Persistence of stream invertebrate communities in relation to environmental variability. *Journal of Animal Ecology*, **56**, 597-613.

Traister EM, McDowell WH, Krám P, Fottová D, Kolaríková K (2013) Persistent effects of acidification on stream ecosystem structure and function. *Freshwater Science*, **32**, 586-596.

Treonis AM, Wall DH, Virginia RA (2002) Field and microcosm studies of decomposition and soil biota in a cold desert soil. *Ecosystems,* **5**, 159–170.

Tylianakis JM, Tscharntke T, Lewis OT (2007) Habitat modification alters the structure of tropical host–parasitoid food webs. *Nature*, **445**, 202-205.

United Kingdom Acid Waters Monitoring Network (UKAWMN) (2010) *UKAWMN 20 Year Interpretative Report: Report to DEFRA* (Eds: M. Kernan, R. W. Battarbee, C. J. Curtis, D. T. Monteith, E. M. Shilland). ENSIS Publishing, London.

Van Gestel CAM, Kruidenier M, Berg MP (2003) Suitability of wheat straw decomposition, cotton strip degradation and bait-lamina feeding tests to determine soil invertebrate activity. *Biology and Fertility of Soils,* **37**, 386.

Van Veen FJ, Morris RJ, Godfray HCJ (2006) Apparent competition, quantitative food webs, and the structure of phytophagous insect communities. *Annual Review of Entomology*, **51**, 187-208.

Vucic-Pestic O, Ehnes RB, Rall BC, Brose U (2011) Warming up the system: higher predator feeding rates but lower energetic efficiencies, *Global Change Biology*, **17**, 1301–1310.

Warren PH, Lawton JH (1987) Invertebrate predator-prey body size relationships: an explanation for upper triangular food webs and patterns in food web structure? *Oecologia*, **74**, 231-235.

Webster JR, Benfield EF (1986) Vascular plant breakdown in freshwater ecosystems. *Annual Review of Ecology and Systematics*, **17**, 567-594.

Wilcock HR, Bruford MW, Nichols RA, Hildrew AG (2007) Landscape, habitat characteristics and the genetic population structure of two caddisflies. *Freshwater Biology*, **52**, 1907-1929.

Williams AC, McCarthy BC (2001) A new index of interspecific competition for replacement and additive designs. *Ecological Research*, **16**, 29-40.

Williams RJ, Martinez ND (2000) Simples rules yield complex food webs, *Nature*, **404,** 180-183.

Williams RJ, Martinez ND (2004) Stabilization of chaotic and non-permanent food-web dynamics. *The European Physical Journal B-Condensed Matter and Complex Systems*, **38**, 297-303.

Williamson MH (1957) An elementary theory of interspecific competition. *Nature*, **180**, 422-25.

Woodward G (2009) Biodiversity, ecosystem functioning and food webs in freshwaters: assembling the jigsaw puzzle. *Freshwater Biology*, **54**, 2171–2187.

Woodward G, Hildrew AG (2001) Invasion of a stream food web by a new top predator. *Journal of Animal Ecology*, **70**, 273 - 288

Woodward G, Hildrew AG (2002a) Differential vulnerability of prey to an invading top predator: integrating field surveys and laboratory experiments. *Ecological Entomology*, **27**, 732 – 744.

Woodward G, Hildrew AG (2002b) Body-size determinants of niche overlap and intraguild predation within a complex food web. *Journal of Animal Ecology*, **71**, 1063 – 1074.

Woodward G, Warren PH (2007). Body size and predatory interactions in freshwaters: Scaling from individuals to communities. In: *Body size: The Structure and Function of Aquatic Ecosystems* (Ed. by A.G. Hildrew, D. Raffaelli and R. Edmonds-Brown), pp. 98–117. Cambridge University Press, Cambridge.

Woodward G, Jones JI, Hildrew AG (2002) Community persistence in Broadstone Stream (U.K.) over three decades. *Freshwater Biology*, **47**, 1419–1435.

Woodward G, Speirs DC, Hildrew AG (2005a) Quantification and resolution of a complex, size-structured food web. *Advances in Ecological Research*, **36**, 85-131.

Woodward G, Ebenman B, Emmerson M, Montoya JM, Olesen JM, Valido A, Warren PH (2005b) Body size in ecological networks. *Trends in Ecology and Evolution*, **20,** 402 – 409.

Woodward G, Papantoniou G, Edwards F, Lauridsen RB (2008) Trophic trickles and cascades in a complex food web: impacts of a keystone predator on stream community structure and ecosystem processes. *Oikos*, **117**, 683-692.

Woodward G, Benstead JP, Beveridge OS, Blanchard J. Brey T, Brown LE, Cross WF, Friberg N, Ings TC, Jacob U, Jennings S, Ledger ME, Milner AM, Montoya JM, O'Gorman E, Oleson JM, Petchey OL, Pichler DE, Reuman DC, Thompson MSA, van Veen FJL, Yvon-Durocher G (2010a) Ecological Networks in a Changing Climate. *Advances in Ecological Research*, **42**, 71-138.

Woodward G, Blanchard J, Lauridsen RB, Edwards FK, Jones JI, Figueroa D, Warren PH, Petchey OL (2010b) Individual-Based Food Webs: Species Identity, Body Size and Sampling Effects. *Advances in Ecological Research*, **43**, 211-266.

Woodward G, Gray C, Baird DJ (2013) Biomonitoring for the 21st Century: new perspectives in an age of globalisation and emerging environmental threats. *Limnetica*, **32**, 159-174.

Wooster D, Sih A (1995) A review of the drift and activity responses of stream prey to predator presence. *Oikos*, **72**, 3-8.

Wright RF, Harriman R, Henriksen A, Morrison B, Caines LA (1980) Acid lakes and streams in the Galloway area, south-western Scotland. In: Drabløs, D., Tollan,A. (Eds.), *Ecological Impact of Acid Precipitation. Proceedings of An International Conference.* Sandefjord, Norway, pp. 248–249.

Yan ND, Leung B, Keller W, Arnott SE, Gunn JM, Raddum GG (2003) Developing conceptual frameworks for the recovery of aquatic biota from acidification. *Ambio*, **32**, 165-169.

Yvon-Durocher G, Montoya JM, Emmerson MC, Woodward G (2008) Macroecological patterns and niche structure in new marine food web. *Central European Journal of Biology*, **3**, 91–103.

Yvon-Durocher G, Reiss J, Blanchard J, Ebenman B, Perkins DM, Reuman DC, Thierry A, Woodward G, Petchey OL (2011) Across ecosystem comparisons of size structure: methods, approaches and prospects. *Oikos*, **120**, 550-563.

Zar JH (1996) *Biostatistical Analysis*, 3rd edition, Prentice Hall, London.

Appendices

Appendix 1. Taxa used in the predator-prey interaction experiments, listed with pH optima, length of time recorded in the Broadstone historical dataset, and densities (individuals m^{-2}) of all prey species in both experimental aquaria and natural environments. *N.pictetii*, *L.nigra* and *P.submarginata* densities are taken from Broadstone Stream, where they are resident, and *B.rhodani* densities are taken from Withyham Stream.

	Name	pH optima	Recorded in Broadstone				
Predators	*Plectrocnemia conspersa* Curtis (Trichoptera: Polycentropodidae)	5	1970s-present	Dominant in 1970s			
	Sialis fuliginosa Pictet (Megaloptera: Sialidae)	5.1	1970s-present	Dominant in 1980s			
	Cordulegaster boltonii Donovan (Anisoptera: Cordulegasteridae)	5.4	1970s-present	Dominant in 1990s			
				Experimental density		**Natural density**	
				Minimum	Maximum	Minimum	Maximum
Prey	*Nemurella pictetii* Klapálek (Plecoptera: Nemouridae)	4.7	1970s-present	43	684	16	1520
	Leuctra nigra Olivier (Plecoptera: Leuctridae)	5	1970s-present	43	684	32	3680
	Paraleptophlebia submarginata Stephens (Ephemeroptera: Leptophlebidae)	5.6	1990s-present	43	1368	0	3
	Baetis rhodani Pictet (Ephermeroptera: Baetidae)	5.9	Absent	43	1368	2	1432

Appendix 2. Akaike information criterion (AICs) comparing two different functional response models (Type II and Type III) for all predator-prey combinations, at three discrete temperature treatments. NA denotes a predator-prey combination where that particular model could not be fitted.

Predator	Prey	Temperature (°C)	AIC	
			Type II	Type III
C.boltonii	*B.rhodani*	5	90.2	79.3
C.boltonii	*B.rhodani*	10	108.3	95.5
C.boltonii	*B.rhodani*	15	111.0	98.2
C.boltonii	*L.nigra*	5	NA	33.9
C.boltonii	*L.nigra*	10	63.1	59.3
C.boltonii	*L.nigra*	15	67.6	62.8
C.boltonii	*N.pictetii*	5	NA	43.1
C.boltonii	*N.pictetii*	10	65.2	60.8
C.boltonii	*N.pictetii*	15	76.8	71.0
C.boltonii	*P.submarginata*	5	103.8	90.3
C.boltonii	*P.submarginata*	10	113.9	94.5
C.boltonii	*P.submarginata*	15	NA	88.7
S.fuliginosa	*B.rhodani*	5	NA	14.8
S.fuliginosa	*B.rhodani*	10	37.4	37.2
S.fuliginosa	*B.rhodani*	15	NA	41.2
S.fuliginosa	*L.nigra*	5	20.0	19.3
S.fuliginosa	*L.nigra*	10	44.4	44.0
S.fuliginosa	*L.nigra*	15	20.0	19.3
S.fuliginosa	*N.pictetii*	5	NA	26.1
S.fuliginosa	*N.pictetii*	10	NA	32.0
S.fuliginosa	*N.pictetii*	15	NA	21.1
S.fuliginosa	*P.submarginata*	5	NA	24.7
S.fuliginosa	*P.submarginata*	10	38.4	37.7
S.fuliginosa	*P.submarginata*	15	NA	44.9
P.conspersa	*B.rhodani*	5	NA	41.9
P.conspersa	*B.rhodani*	10	44.7	43.7
P.conspersa	*B.rhodani*	15	NA	51.1
P.conspersa	*L.nigra*	5	46.7	46.0
P.conspersa	*L.nigra*	10	51.7	50.9
P.conspersa	*L.nigra*	15	52.3	52.0
P.conspersa	*N.pictetii*	5	NA	32.0
P.conspersa	*N.pictetii*	10	NA	40.7
P.conspersa	*N.pictetii*	15	NA	64.1
P.conspersa	*P.submarginata*	5	NA	39.4
P.conspersa	*P.submarginata*	10	NA	48.0
P.conspersa	*P.submarginata*	15	NA	53.1

Appendix 3. Natural biomass (mg), experimental saturation points and 95% confidence intervals for functional responses at all predator-prey pairwise combinations, once converted into biomass consumed (in milligrams).

Predator	Prey species	Natural biomass (mg)			Biomass saturation point (mg)	95% CIs
		Minimum	Maximum	Mean		
C.boltonii	*L.nigra*	0.44	50.71	9.99	2.43	1.34, 6.35
	N.pictetii	0.14	13.04	1.1	1.65	1.16, 3.54
	B.rhodani	0.02	12.57	1.01	3.06	2.64, 3.56
	P.submarginata	0	0.02	0.01	2.24	2.12, 2.76
S.fuliginosa	*L.nigra*	0.44	50.71	9.99	N/A	N/A
	N.pictetii	0.14	13.04	1.1	0.11	0.01, 0.18
	B.rhodani	0.02	12.57	1.01	1.49	0.29, 1.52
	P.submarginata	0	0.02	0.01	0.28	0.02, 2.11
P.conspersa	*L.nigra*	0.44	50.71	9.99	0.72	0.45, 1.16
	N.pictetii	0.14	13.04	1.1	0.73	0.43, 2.03
	B.rhodani	0.02	12.57	1.01	0.48	0.28, 0.58
	P.submarginata	0	0.02	0.01	0.53	0.31, 0.82

Appendix 4 Parameters and standard errors (SE) estimated by the Type III functional response model. ***a*** = attack rate, and ***h*** = handling time. (Level of significance: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$).

Predator	Prey	Temperature (°C)	*a*	SE	*h*	SE
C.boltonii	*B.rhodani*	5	0.09***	0.02	0.15***	0.02
C.boltonii	*B.rhodani*	10	0.20***	0.05	0.12***	0.01
C.boltonii	*B.rhodani*	15	0.27***	0.06	0.10***	0.01
C.boltonii	*L.nigra*	5	0.01*	0.005	0.25	0.47
C.boltonii	*L.nigra*	10	0.09*	0.03	0.29***	0.07
C.boltonii	*L.nigra*	15	0.12*	0.04	0.27***	0.06
C.boltonii	*N.pictetii*	5	0.02*	0.001	0.18	0.17
C.boltonii	*N.pictetii*	10	0.18*	0.04	0.25***	0.06
C.boltonii	*N.pictetii*	15	0.22***	0.07	0.21***	0.03
C.boltonii	*P.submarginata*	5	0.12***	0.02	0.12***	0.02
C.boltonii	*P.submarginata*	10	0.21***	0.06	0.10***	0.01
C.boltonii	*P.submarginata*	15	0.38***	0.07	0.09***	0.007
S.fuliginosa	*B.rhodani*	5	0.01	0.003	2.25	8.26
S.fuliginosa	*B.rhodani*	10	0.10	0.10	1.40	1.45
S.fuliginosa	*B.rhodani*	15	0.01	0.01	0.73	0.46
S.fuliginosa	*L.nigra*	5	0.003	0.004	2.03	3.38
S.fuliginosa	*L.nigra*	10	0.06	0.05	1.31*	0.53
S.fuliginosa	*L.nigra*	15	0.07	0.01	0.03	5.94
S.fuliginosa	*N.pictetii*	5	0.01	0.01	0.95	1.17
S.fuliginosa	*N.pictetii*	10	0.01	0.001	0.81	0.67
S.fuliginosa	*N.pictetii*	15	0.02	0.002	0.48	2.51
S.fuliginosa	*P.submarginata*	5	0.03	0.03	0.49	1.72
S.fuliginosa	*P.submarginata*	10	0.02	0.01	1.07	0.59
S.fuliginosa	*P.submarginata*	15	0.03	0.02	0.62*	0.28
P.conspersa	*B.rhodani*	5	0.05	0.04	1.13**	0.43
P.conspersa	*B.rhodani*	10	0.08	0.06	1.06**	0.38
P.conspersa	*B.rhodani*	15	0.07	0.05	0.79**	0.27
P.conspersa	*L.nigra*	5	0.09	0.08	1.33**	0.49
P.conspersa	*L.nigra*	10	0.04	0.03	0.69*	0.27
P.conspersa	*L.nigra*	15	0.06	0.03	0.59**	0.21
P.conspersa	*N.pictetii*	5	0.01	0.009	0.84	0.67
P.conspersa	*N.pictetii*	10	0.04	0.02	0.47*	0.20
P.conspersa	*N.pictetii*	15	0.13*	0.06	0.37***	0.09
P.conspersa	*P.submarginata*	5	0.02	0.01	1.08	0.59
P.conspersa	*P.submarginata*	10	0.05	0.02	0.83*	0.31
P.conspersa	*P.submarginata*	15	0.06	0.04	0.63**	0.22